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(54) Title: TACI ANTIBODIES AND USES THEREOF

(57) Abstract: TACI receptor antibodies are provided. The TACI antibodies may be included in pharmaceutical compositions, articles of manufacture, or kits. Methods of treatment and diagnosis using the TACI antibodies are also provided.

TACI ANTIBODIES AND USES THEREOF

FIELD OF THE INVENTION

This invention relates generally to TACI antibodies, and to methods of using TACI antibodies to modulate for example, activity of TACI, tumor necrosis factor (TNF) and TNFR-related molecules, including members of the TNF and TNFR families referred to as TALL-1, APRIL, TACI, BR3, and BCMA. The invention also relates to methods for *in vitro*, *in situ*, and/or *in vivo* diagnosis and/or treatment of mammalian cells or pathological conditons associated with such TNF and TNFR-related molecules.

BACKGROUND OF THE INVENTION

Various molecules, such as tumor necrosis factor-alpha ("TNF-alpha"), tumor necrosis factor-beta ("TNF-beta" or "lymphotoxin-alpha"), lymphotoxin-beta ("LT-beta"), CD30 ligand, CD27 ligand, CD40 ligand, OX-40 ligand, 4-1BB ligand, Apo-1 ligand (also referred to as Fas ligand or CD95 ligand), Apo-2 ligand (also referred to as Apo2L or TRAIL), Apo-3 ligand (also referred to as TWEAK), APRIL, OPG ligand (also referred to as RANK ligand, ODF, or TRANCE), and TALL-1 (also referred to as BlyS, BAFF or THANK) have been identified as members of the tumor necrosis factor ("TNF") family of cytokines [See, e.g., Gruss and Dower, Blood, 85:3378-3404 (1995); Schmid et al., Proc. Natl. Acad. Sci., 83:1881 (1986); Dealtry et al., Eur. J. Immunol., 17:689 (1987); Pitti et al., J. Biol. Chem., 271:12687-12690 (1996); Wiley et al., Immunity, 3:673-682 (1995); Browning et al., Cell, 72:847-856 (1993); Armitage et al., Nature, 357:80-82 (1992), WO 97/01633 published January 16, 1997; WO 97/25428 published July 17, 1997; Marsters et al., Curr. Biol., 8:525-528 (1998); Chicheportiche et al., Biol. Chem., 272:32401-32410 (1997); Hahne et al., J. Exp. Med., 188:1185-1190 (1998); WO98/28426 published July 2, 1998; WO98/46751 published October 22, 1998; WO/98/18921 published May 7, 1998; Moore et al., Science, 285:260-263 (1999); Shu et al., J. Leukocyte Biol., 65:680 (1999); Schneider et al., J. Exp. Med., 189:1747-1756 (1999); Mukhopadhyay et al., J. Biol. Chem., 274:15978-15981 (1999)]. Among these molecules, TNF-alpha, TNF-beta, CD30 ligand, 4-1BB ligand, Apo-1 ligand, Apo-2 ligand (Apo2L/TRAIL) and Apo-3 ligand (TWEAK) have been reported to be involved in apoptotic cell death.

Various molecules in the TNF family also have purported role(s) in the function or development of the immune system [Gruss et al., Blood, 85:3378 (1995)]. Zheng et al. have reported that TNF is involved in post-stimulation apoptosis of CD8-positive T cells [Zheng et al., Nature, 377:348-351 (1995)]. Other investigators have reported that CD30 ligand may be involved in deletion of self-reactive T cells in the thymus [Amakawa et al., Cold Spring Harbor Laboratory Symposium on Programmed Cell Death, Abstr. No. 10, (1995)]. CD40 ligand activates many functions of B cells, including proliferation, immunoglobulin secretion, and survival [Renshaw et al., J. Exp. Med., 180:1889 (1994)]. Another recently identified TNF family cytokine, TALL-1 (BlyS), has been reported, under certain conditions, to induce B cell proliferation and immunoglobulin secretion. [Moore et al., supra; Schneider et al., supra; Mackay et al., J. Exp. Med., 190:1697 (1999); Shu et al., J. Leukocyte Biol., 65:680-683 (1999); Gross et al., Nature, 404:995-999 (2000)].

Mutations in the mouse Fas/Apo-1 receptor or ligand genes (called *lpr* and *gld*, respectively) have been associated with some autoimmune disorders, indicating that Apo-1 ligand may play a role in regulating the clonal deletion of self-reactive lymphocytes in the periphery [Krammer et al., Curr. Op. Immunol., 6:279-289 (1994); Nagata et al., Science, 267:1449-1456 (1995)]. Apo-1 ligand is also reported to induce post-stimulation apoptosis in CD4-positive T lymphocytes and in B lymphocytes, and may be involved in the elimination of activated lymphocytes when their function is no longer needed [Krammer et al., supra; Nagata et al., supra]. Agonist mouse monoclonal antibodies specifically binding to the Apo-1 receptor have been reported to exhibit cell killing activity that is comparable to or similar to that of TNF- α [Yonehara et al., J. Exp. Med., 169:1747-1756 (1989)].

The TNF-related ligand called OPG ligand (also referred to as RANK ligand, TRANCE, or ODF) has been reported in the literature to have some involvement in certain immunoregulatory activities. WO98/28426 published July 2, 1998 describes the ligand (referred to therein as RANK ligand) as a Type 2 transmembrane protein, which in a soluble form, was found to induce maturation of dendritic cells,

enhance CD1a⁺ dendritic cell allo-stimulatory capacity in a MLR, and enhance the number of viable human peripheral blood T cells *in vitro* in the presence of TGF-beta. [see also, Anderson et al., Nature, 390:175-179 (1997)]. The WO98/28426 reference also

5 discloses that the ligand enhanced production of TNF-alpha by one macrophage tumor cell line (called RAW264.7; ATCC TIB71), but did not stimulate nitric oxide production by those tumor cells.

The putative roles of OPG ligand/TRANCE/ODF in modulating dendritic cell activity [see, e.g., Wong et al., J. Exp. Med., 10 186:2075-2080 (1997); Wong et al., J. Leukocyte Biol., 65:715-724 (1999); Josien et al., J. Immunol., 162:2562-2568 (1999); Josien et al., J. Exp. Med., 191:495-501 (2000)] and in influencing T cell activation in an immune response [see, e.g., Bachmann et al., J. Exp. Med., 189:1025-1031 (1999); Green et al., J. Exp. Med., 15 189:1017-1020 (1999)] have been explored in the literature. Kong et al., Nature, 397:315-323 (1999) report that mice with a disrupted *opgl* gene showed severe osteoporosis, lacked osteoclasts, and exhibited defects in early differentiation of T and B lymphocytes. Kong et al. have further reported that systemic 20 activation of T cells *in vivo* led to an OPGL-mediated increase in osteoclastogenesis and bone loss. [Kong et al., Nature, 402:304-308 (1999)].

Induction of various cellular responses mediated by such TNF family cytokines is believed to be initiated by their binding to 25 specific cell receptors. Previously, two distinct TNF receptors of approximately 55-kDa (TNFR1) and 75-kDa (TNFR2) were identified [Hohman et al., J. Biol. Chem., 264:14927-14934 (1989); Brockhaus et al., Proc. Natl. Acad. Sci., 87:3127-3131 (1990); EP 417,563, published March 20, 1991; Loetscher et al., Cell, 61:351 (1990); 30 Schall et al., Cell, 61:361 (1990); Smith et al., Science, 248:1019-1023 (1990); Lewis et al., Proc. Natl. Acad. Sci., 88:2830-2834 (1991); Goodwin et al., Mol. Cell. Biol., 11:3020-3026 (1991)]. Those TNFRs were found to share the typical structure of cell surface receptors including extracellular, transmembrane and 35 intracellular regions. The extracellular portions of both receptors were found naturally also as soluble TNF-binding proteins [Nophar, Y. et al., EMBO J., 9:3269 (1990); and Kohno, T. et al., Proc. Natl. Acad. Sci. U.S.A., 87:8331 (1990); Hale et al., J.

Cell. Biochem. Supplement 15F, 1991, p. 113 (P424)].

The extracellular portion of type 1 and type 2 TNFRs (TNFR1 and TNFR2) contains a repetitive amino acid sequence pattern of four cysteine-rich domains (CRDs) designated 1 through 4, starting
5 from the NH₂-terminus. [Schall et al., supra; Loetscher et al., supra; Smith et al., supra; Nophar et al., supra; Kohno et al., supra; Banner et al., Cell, 73:431-435 (1993)]. A similar repetitive pattern of CRDs exists in several other cell-surface proteins, including the p75 nerve growth factor receptor (NGFR)
10 [Johnson et al., Cell, 47:545 (1986); Radeke et al., Nature, 325:593 (1987)], the B cell antigen CD40 [Stamenkovic et al., EMBO J., 8:1403 (1989)], the T cell antigen OX40 [Mallet et al., EMBO J., 9:1063 (1990)] and the Fas antigen [Yonehara et al., supra and Itoh et al., Cell, 66:233-243 (1991)]. CRDs are also found in the
15 soluble TNFR (sTNFR)-like T2 proteins of the Shope and myxoma poxviruses [Upton et al., Virology, 160:20-29 (1987); Smith et al., Biochem. Biophys. Res. Commun., 176:335 (1991); Upton et al., Virology, 184:370 (1991)]. Optimal alignment of these sequences indicates that the positions of the cysteine residues are well
20 conserved. These receptors are sometimes collectively referred to as members of the TNF/NGF receptor superfamily.

The TNF family ligands identified to date, with the exception of lymphotoxin- α , are typically type II transmembrane proteins, whose C-terminus is extracellular. In contrast, most receptors in
25 the TNF receptor (TNFR) family identified to date are typically type I transmembrane proteins. In both the TNF ligand and receptor families, however, homology identified between family members has been found mainly in the extracellular domain ("ECD"). Several of the TNF family cytokines, including TNF- α , Apo-1 ligand and CD40
30 ligand, are cleaved proteolytically at the cell surface; the resulting protein in each case typically forms a homotrimeric molecule that functions as a soluble cytokine. TNF receptor family proteins are also usually cleaved proteolytically to release soluble receptor ECDs that can function as inhibitors of the
35 cognate cytokines.

The TNFR family member, referred to as RANK, has been identified as a receptor for OPG ligand (see WO98/28426 published

July 2, 1998; Anderson et al., Nature, 390:175-179 (1997); Lacey et al., Cell, 93:165-176 (1998). Another TNFR-related molecule, called OPG (FDCR-1 or OCIF), has also been identified as a receptor for OPG ligand. [Simonet et al., Cell, 89:309 (1997); Yasuda et al., Endocrinology, 139:1329 (1998); Yun et al., J. Immunol., 161:6113-6121 (1998)]. Yun et al., supra, disclose that OPG/FDCR-1/OCIF is expressed in both a membrane-bound form and a secreted form and has a restricted expression pattern in cells of the immune system, including dendritic cells, EBV-transformed B cell lines and tonsillar B cells. Yun et al. also disclose that in B cells and dendritic cells, expression of OPG/FDCR-1/OCIF can be up-regulated by CD40, a molecule involved in B cell activation. However, Yun et al. acknowledge that how OPG/FDCR-1/OCIF functions in the regulation of the immune response is unknown.

More recently, other members of the TNFR family have been identified. In von Bulow et al., Science, 278:138-141 (1997), investigators describe a plasma membrane receptor referred to as Transmembrane Activator and CAML-Interactor or "TACI". The TACI receptor is reported to contain a cysteine-rich motif characteristic of the TNFR family. In an in vitro assay, cross linking of TACI on the surface of transfected Jurkat cells with TACI-specific antibodies led to activation of NF-KB. [see also, WO 98/39361 published September 18, 1998]. TACI knockout mice have been reported to have hyperresponsive B cells, while BCMA null mice had no discernable phenotype [Yan et al., Nature Immunology, 2:638-643 (2001); von Bulow et al., Immunity, 14:573-582 (2001); Xu et al., Mol. Cell. Biology, 21:4067-4074 (2001)]. See also, WO 00/40716 published July 13, 2000; WO 01/85782 published November 15, 2001.

Laabi et al., EMBO J., 11:3897-3904 (1992) reported identifying a new gene called "BCM" whose expression was found to coincide with B cell terminal maturation. The open reading frame of the BCM normal cDNA predicted a 184 amino acid long polypeptide with a single transmembrane domain. These investigators later termed this gene "BCMA." [Laabi et al., Nucleic Acids Res., 22:1147-1154 (1994)]. BCMA mRNA expression was reported to be absent in human malignant B cell lines which represent the pro-B lymphocyte stage, and thus, is believed to be linked to the stage

of differentiation of lymphocytes [Gras et al., Int. Immunology, 7:1093-1106 (1995)]. In Madry et al., Int. Immunology, 10:1693-1702 (1998), the cloning of murine BCMA cDNA was described. The murine BCMA cDNA is reported to encode a 185 amino acid long
5 polypeptide having 62% identity to the human BCMA polypeptide. Alignment of the murine and human BCMA protein sequences revealed a conserved motif of six cysteines in the N-terminal region, suggesting that the BCMA protein belongs to the TNFR superfamily [Madry et al., supra]. See also, WO 00/68378 published November
10 16, 2000; WO 00/50633 published August 31, 2000.

The Tall-1 (BlyS) ligand has been reported to bind the TACI and BCMA receptors [Gross et al., supra, (2000); Thompson et al., J. Exp. Med., 192:129-135 (2000); Yan et al., supra, (2000); Marsters et al., Curr. Biol., 10:785-758 (2000); WO 00/40716
15 published July 13, 2000; WO 00/67034 published November 9, 2000; WO 01/12812 published February 22, 2001]. TACI and BCMA have likewise been reported to bind to the ligand known as April.

In Marsters et al., Curr. Biol., 6:750 (1996), investigators describe a full length native sequence human polypeptide, called
20 Apo-3, which exhibits similarity to the TNFR family in its extracellular cysteine-rich repeats and resembles TNFR1 and CD95 in that it contains a cytoplasmic death domain sequence [see also Marsters et al., Curr. Biol., 6:1669 (1996)]. Apo-3 has also been referred to by other investigators as DR3, wsl-1, TRAMP, and LARD
25 [Chinnaiyan et al., Science, 274:990 (1996); Kitson et al., Nature, 384:372 (1996); Bodmer et al., Immunity, 6:79 (1997); Screaton et al., Proc. Natl. Acad. Sci., 94:4615-4619 (1997)].

Pan et al. have disclosed another TNF receptor family member referred to as "DR4" [Pan et al., Science, 276:111-113 (1997); see
30 also WO98/32856 published July 30, 1998]. The DR4 was reported to contain a cytoplasmic death domain capable of engaging the cell suicide apparatus. Pan et al. disclose that DR4 is believed to be a receptor for the ligand known as Apo2L/TRAIL.

In Sheridan et al., Science, 277:818-821 (1997) and Pan et
35 al., Science, 277:815-818 (1997), another molecule believed to be a receptor for Apo2L/TRAIL is described [see also, WO98/51793 published November 19, 1998; WO98/41629 published September 24, 1998]. That molecule is referred to as DR5 (it has also been

alternatively referred to as Apo-2; TRAIL-R, TR6, Tango-63, hAPO8, TRICK2 or KILLER [Screaton et al., Curr. Biol., 7:693-696 (1997); Walczak et al., EMBO J., 16:5386-5387 (1997); Wu et al., Nature Genetics, 17:141-143 (1997); WO98/35986 published August 20, 1998; EP870,827 published October 14, 1998; WO98/46643 published October 22, 1998; WO99/02653 published January 21, 1999; WO99/09165 published February 25, 1999; WO99/11791 published March 11, 1999]. Like DR4, DR5 is reported to contain a cytoplasmic death domain and be capable of signaling apoptosis. The crystal structure of the complex formed between Apo-2L/TRAIL and DR5 is described in Hymowitz et al., Molecular Cell, 4:563-571 (1999).

Yet another death domain-containing receptor, DR6, was recently identified [Pan et al., FEBS Letters, 431:351-356 (1998)]. Aside from containing four putative extracellular cysteine rich domains and a cytoplasmic death domain, DR6 is believed to contain a putative leucine-zipper sequence that overlaps with a proline-rich motif in the cytoplasmic region. The proline-rich motif resembles sequences that bind to src-homology-3 domains, which are found in many intracellular signal-transducing molecules. In contrast to other death domain-containing receptors referred to above, DR6 does not induce cell death in the apoptosis sensitive indicator cell line, MCF-7, suggesting an alternate function for this receptor. Consistent with this observation, DR6 is presently believed not to associate with death-domain containing adapter molecules, such as FADD, RAIDD and RIP, that mediate downstream signaling from activated death receptors [Pan et al., FEBS Lett., 431:351 (1998)].

A further group of recently identified receptors are referred to as "decoy receptors," which are believed to function as inhibitors, rather than transducers of signaling. This group includes DCR1 (also referred to as TRID, LIT or TRAIL-R3) [Pan et al., Science, 276:111-113 (1997); Sheridan et al., Science, 277:818-821 (1997); McFarlane et al., J. Biol. Chem., 272:25417-25420 (1997); Schneider et al., FEBS Letters, 416:329-334 (1997); Degli-Esposti et al., J. Exp. Med., 186:1165-1170 (1997); and Mongkolsapaya et al., J. Immunol., 160:3-6 (1998)] and DCR2 (also called TRUNDD or TRAIL-R4) [Marsters et al., Curr. Biol., 7:1003-1006 (1997); Pan et al., FEBS Letters, 424:41-45 (1998); Degli-

Esposti et al., Immunity, 7:813-820 (1997)], both cell surface molecules, as well as OPG [Simonet et al., supra; Emery et al., infra] and DCR3 [Pitti et al., Nature, 396:699-703 (1998)], both of which are secreted, soluble proteins.

5 Additional newly identified members of the TNFR family include CAR1, HVEM, GITR, ZTNFR-5, NTR-1, and TNFL1 [Brojatsch et al., Cell, 87:845-855 (1996); Montgomery et al., Cell, 87:427-436 (1996); Marsters et al., J. Biol. Chem., 272:14029-14032 (1997); Nocentini et al., Proc. Natl. Acad. Sci. USA 94:6216-6221 (1997);
10 Emery et al., J. Biol. Chem., 273:14363-14367 (1998); WO99/04001 published January 28, 1999; WO99/07738 published February 18, 1999; WO99/33980 published July 8, 1999].

As reviewed recently by Tewari et al., TNFR1, TNFR2 and CD40 modulate the expression of proinflammatory and costimulatory
15 cytokines, cytokine receptors, and cell adhesion molecules through activation of the transcription factor, NF- κ B [Tewari et al., Curr. Op. Genet. Develop., 6:39-44 (1996)]. NF- κ B is the prototype of a family of dimeric transcription factors whose subunits contain conserved Rel regions [Verma et al., Genes Develop., 9:2723-2735
20 (1996); Baldwin, Ann. Rev. Immunol., 14:649-681 (1996)]. In its latent form, NF- κ B is complexed with members of the I κ B inhibitor family; upon inactivation of the I κ B in response to certain stimuli, released NF- κ B translocates to the nucleus where it binds to specific DNA sequences and activates gene transcription. As
25 described above, the TNFR members identified to date either include or lack an intracellular death domain region. Some TNFR molecules lacking a death domain, such as TNFR2, CD40, HVEM, and GITR, are capable of modulating NF- κ B activity. [see, e.g., Lotz et al., J. Leukocyte Biol., 60:1-7 (1996)].

30 For a review of the TNF family of cytokines and their receptors, see Ashkenazi and Dixit, Science, 281:1305-1308 (1998); Golstein, Curr. Biol., 7:750-753 (1997); Gruss and Dower, supra, and Nagata, Cell, 88:355-365 (1997); Locksley et al., Cell, 104:487-501 (2001); Wallach, TNF Ligand & TNF/NGF Receptor Families, Cytokine Reference, Academic Press, pp.371-411 (2001).
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SUMMARY OF THE INVENTION

The present invention provides TACI antibodies and methods for using TACI antibodies. The antibodies may act as antagonists or agonists, and find utility for, among other things, *in vitro*, *in situ*, or *in vivo* diagnosis or treatment of mammalian cells or pathological conditions associated with the presence (or absence) of TALL-1, APRIL, TACI, BCMA, TACIs, or BR3.

Preferred embodiments of the invention include anti-TACI antibodies which are capable of specifically binding to human TACI and/or are capable of modulating biological activities associated with TACI and/or its ligand(s), and thus are useful in the treatment of various diseases and pathological conditions such as immune related diseases.

In one embodiment of the invention, the anti-TACI antibodies activate TACI. In another embodiment, anti-TACI antibodies inhibit B-cell proliferation or survival with or without blocking Blys binding to TACI. In another embodiment, present invention provides methods for the use of TACI antibodies to block or neutralize the interaction between TALL-1 or April and TACI. Such antagonists may also block or neutralize the interaction between TALL-1 and TACI and/or BCMA. For example, the invention provides a method comprising exposing a mammalian cell, such as a white blood cell (preferably a B cell), to one or more TACI antibodies in an amount effective to decrease, neutralize or block activity of the TALL-1 ligand or the TACI receptor. The cell may be in cell culture or in a mammal, e.g. a mammal suffering from, for instance, an immune related disease or cancer.

Typical methods of the invention include methods to treat pathological conditions or diseases in mammals associated with or resulting from increased or enhanced TALL-1 or APRIL expression and/or activity. In the methods of treatment, TACI antibodies may be administered which preferably block or reduce the respective receptor binding or activation by TALL-1 ligand and/or APRIL ligand. Optionally, the TACI antibodies employed in the methods will be capable of blocking or neutralizing the activity of both TALL-1 and APRIL, e.g., a dual antagonist which blocks or neutralizes activity of both TALL-1 and APRIL. Optionally, the antagonist molecule(s) employed in the methods will be capable of

blocking or neutralizing the activity of TALL-1 but not APRIL. The methods contemplate the use of a single type of antagonist molecule or a combination of two or more types of antagonist.

The invention also provides compositions which comprise TACI
5 antibodies. Optionally, the compositions of the invention will include pharmaceutically acceptable carriers or diluents. Preferably, the compositions will include one or more TACI antibodies in an amount which is therapeutically effective to treat a pathological condition or disease.

10 The invention also provides articles of manufacture and kits which include one or more TACI antibodies.

In more particular embodiments, there are provided antibodies which specifically bind to a TACI receptor comprising amino acids 2 to 166 of SEQ ID NO: 3. Optionally, the antibody does not bind
15 BCMA receptor, and is a monoclonal antibody. Optionally, the monoclonal antibody comprises the 1G10.1.5 antibody secreted by the hybridoma deposited with ATCC as accession number PTA-4297; the 5B6.3.10 antibody secreted by the hybridoma deposited with ATCC as accession number PTA-4298, or the 6D11.3.1 antibody secreted by the
20 hybridoma deposited with ATCC as accession number PTA-4299.

Also provided are monoclonal antibodies which bind to the same epitope as the epitope to which the 1G10.1.5 monoclonal antibody produced by the hybridoma cell line deposited as ATCC accession number PTA-4297 binds; the 5B6.3.10 monoclonal antibody produced by
25 the hybridoma cell line deposited as ATCC accession number PTA-4298 binds; the 6D11.3.1 monoclonal antibody produced by the hybridoma cell line deposited as ATCC accession number PTA-4299 binds, the antibody produced by the 7B6.15.11 hybridoma cell line deposited as ATCC accession number PTA-5000 binds or the antibody produced by
30 the 4C7.2.1 hybridoma deposited with ATCC as accession number PTA-4999 binds.

In yet other particular embodiments, there is provided the hybridoma cell line which produces monoclonal antibody 1G10.1.5 and deposited with ATCC as accession number PTA-4297, the monoclonal
35 antibody 1G10.1.5 secreted by the hybridoma deposited with ATCC as accession number PTA-4297, the hybridoma cell line which produces monoclonal antibody 5B6.3.10 and deposited with ATCC as accession number PTA-4298, the monoclonal antibody 5B6.3.10 secreted by the

hybridoma deposited with ATCC as accession number PTA-4298, the hybridoma cell line which produces monoclonal antibody 6D11.3.1 and deposited with ATCC as accession number PTA-4299, the monoclonal antibody 6D11.3.1 secreted by the hybridoma deposited with ATCC as
5 accession number PTA-4299; hybridoma cell line which produces the monoclonal antibody G10.1.5 and deposited with ATCC as accession number PTA-4297 and the monoclonal antibody G10.1.5 secreted by the hybridoma deposited with ATCC as accession number PTA-4297; the 7B6.15.11 hybridoma cell line which produces a monoclonal antibody
10 and is deposited with ATCC as accession number PTA-5000, the monoclonal antibody produced by the 7B6.15.11 hybridoma deposited with ATCC as accession number PTA-5000; and the 4C7.2.1 hybridoma cell line which produces a monoclonal antibody and is deposited with ATCC as accession number PTA-4999, the monoclonal antibody
15 produced by the 4C7.2.1 hybridoma deposited with ATCC as accession number PTA-4999.

There are also provided isolated anti-TACI receptor monoclonal antibodies, comprising antibodies which bind to TACI receptor comprising amino acids 2 to 166 of SEQ ID NO: 3 and competitively
20 inhibit binding of the monoclonal antibody produced by the hybridoma deposited as ATCC PTA-4297 to said TACI receptor; isolated anti-TACI receptor monoclonal antibodies, comprising antibodies which bind to TACI receptor comprising amino acids 2 to 166 of SEQ ID NO: 3 and competitively inhibit binding of the
25 monoclonal antibody produced by the hybridoma deposited as ATCC PTA-4298 to said TACI receptor; and isolated anti-TACI receptor monoclonal antibodies, comprising antibodies which bind to TACI receptor comprising amino acids 2 to 166 of SEQ ID NO: 3 and competitively inhibit binding of the monoclonal antibody produced
30 by the hybridoma deposited as ATCC PTA-4299 to said TACI receptor.

In yet another embodiment, the antibodies are chimeric anti-TACI antibodies which specifically bind to TACI polypeptide and comprise (a) a sequence derived from the 1G10.1.5 antibody secreted by the hybridoma deposited with ATCC as accession number PTA-4297;
35 (b) a sequence derived from the 5B6.3.10 antibody secreted by the hybridoma deposited with ATCC as accession number PTA-4298; (c) a sequence derived from the 6D11.3.1 antibody secreted by the hybridoma deposited with ATCC as accession number PTA-4299; (d) a

sequence derived from the antibody secreted by the 7B6.15.11 hybridoma deposited with ATCC as accession number PTA-5000. Optionally, such antibodies are humanized antibodies or (e) a sequence derived from the antibody secreted by the 4C7.2.1 hybridoma deposited with ATCC as accession number PTA-4999.

In another embodiment, the anti-TACI receptor antibodies are linked to one or more non-proteinaceous polymers selected from the group consisting of polyethylene glycol, polypropylene glycol, and polyoxyalkylene, or to a cytotoxic agent or enzyme, or to a radioisotope, fluorescent compound or chemiluminescent compound.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B show a polynucleotide sequence encoding a native sequence human TACI (SEQ ID NO:1) (reverse complimentary sequence is provided in SEQ ID NO:2) and its putative amino acid sequence (SEQ ID NO:3). Figure 1C shows a TACI spliced variant referred to as "hTACI (265)" (SEQ ID NO:17).

Figure 2 shows a polynucleotide sequence encoding a native sequence human BCMA (SEQ ID NO:4) (reverse complimentary sequence is provided in SEQ ID NO:5) and its putative amino acid sequence (SEQ ID NO:6).

Figure 3 shows a polynucleotide sequence encoding a native sequence human TALL-1 (SEQ ID NO:7) (reverse complimentary sequence is provided in SEQ ID NO:8) and its putative amino acid sequence (SEQ ID NO:9).

Figures 4A-4B show a polynucleotide sequence encoding a native sequence human APRIL (SEQ ID NO:10) (reverse complimentary sequence is provided in SEQ ID NO:11) and its putative amino acid sequence (SEQ ID NO:12).

Figure 5A shows a polynucleotide sequence (start and stop codons are underlined) encoding a native sequence human TACIs (SEQ ID NO:13) and Figure 5B shows its putative amino acid sequence (SEQ ID NO:14).

Figure 6A shows a polynucleotide sequence (start and stop codons are underlined) encoding a native sequence human BR3 (SEQ ID NO:15), and Figure 6B shows its putative amino acid sequence (SEQ ID NO:16).

Figures 7A-7B show exemplary methods for calculating the %

amino acid sequence identity of the amino acid sequence designated "Comparison Protein" to the amino acid sequence designated "PRO". For purposes herein, the "PRO" sequence may be the TACI, BCMA, TALL-1, APRIL, TACIs, or BR3 sequences referred to in the Figures
5 herein.

Figure 8 shows the results of an ELISA assay which examines the ability of antibodies 1D10, 1G10, 5B6 and 6D11 to bind TACI-IgG, BCMA-IgG and CD4-IgG (Control).

Figure 9 is a graph representating data showing that TACI is a
10 negative regulator of TALL-1 stimulation. Figure 9 shows that anti-TACI antibodies 5B6 and 6D11 block B lymphocyte proliferation.

Figure 10 shows the results of a FACS analysis showing that anti-TACI mAbs recognize and bind IM9 cells expressing TACI.

Figure 11 shows (A) three monoclonal antibodies generated in
15 mouse to human TACI (6D11, 7B6, and 4C7) bind to 293 cells transfected with 0.1 µg full-length human TACI for 24 hr and analyzed by FACS using a PE-conjugated α-mouse IgG1 secondary antibody. Isotype control is shown in gray; (B) Activation of NF-κB activity in human 293 cells transfected with full-length human TACI
20 expression plasmid along with 1 µg of ELAM-luciferase reporter plasmid and 0.1 µg control pRL-TK plasmid and then treated with soluble recombinant human BLyS or TACI antibodies, 6D11, 7B6 and 4C7; and (C) inhibition of anti-CD40 antibody/IL-4- induced B-cell proliferation by 6D11 and 7B6 anti-TACI antibodies

25

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

The terms "BR3", "BR3 polypeptide" or "BR3 receptor" when used herein encompass "native sequence BR3 polypeptides" and "BR3
30 variants" (which are further defined herein). "BR3" is a designation given to those polypeptides which are encoded by the nucleic acid molecules comprising the polynucleotide sequences shown in Figure 6 and variants or fragments thereof, nucleic acid molecules comprising the sequence shown in the Figure 6 and
35 variants thereof as well as fragments of the above. The BR3 polypeptides of the invention may be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant and/or synthetic methods.

A "native sequence" BR3 polypeptide comprises a polypeptide having the same amino acid sequence as the corresponding BR3 polypeptide derived from nature. Such native sequence BR3 polypeptides can be isolated from nature or can be produced by recombinant and/or synthetic means. The term "native sequence BR3 polypeptide" specifically encompasses naturally-occurring truncated or secreted forms (e.g., an extracellular domain sequence), naturally-occurring variant forms (e.g., alternatively spliced forms) and naturally-occurring allelic variants of the polypeptide. The BR3 polypeptides of the invention include the BR3 polypeptide comprising or consisting of the contiguous sequence of amino acid residues 1 to 184 of Fig. 6B (SEQ ID NO:16) and the polypeptides described in WO 02/24909 published March 28, 2002 (referred to therein as "BAFF-R").

A BR3 "extracellular domain" or "ECD" refers to a form of the BR3 polypeptide which is essentially free of the transmembrane and cytoplasmic domains. Ordinarily, a BR3 polypeptide ECD will have less than about 1% of such transmembrane and/or cytoplasmic domains and preferably, will have less than about 0.5% of such domains. It will be understood that any transmembrane domain(s) identified for the BR3 polypeptides of the present invention are identified pursuant to criteria routinely employed in the art for identifying that type of hydrophobic domain. The exact boundaries of a transmembrane domain may vary but most likely by no more than about 5 amino acids at either end of the domain as initially identified. ECD forms of BR3 include those comprising amino acids 1 to 77 or 2 to 62 of Figure 6B.

"BR3 variant" means a BR3 polypeptide having at least about 80% amino acid sequence identity with the amino acid sequence of a native sequence full length BR3 or BR3 ECD. Optionally, the BR3 variant includes a single cysteine rich domain. Such BR3 variant polypeptides include, for instance, BR3 polypeptides wherein one or more amino acid residues are added, or deleted, at the N- and/or C-terminus, as well as within one or more internal domains, of the full-length amino acid sequence. Fragments of the BR3 ECD are also contemplated. Ordinarily, a BR3 variant polypeptide will have at least about 80% amino acid sequence identity, more preferably at least about 81% amino acid sequence identity, more preferably at

least about 82% amino acid sequence identity, more preferably at
least about 83% amino acid sequence identity, more preferably at
least about 84% amino acid sequence identity, more preferably at
least about 85% amino acid sequence identity, more preferably at
5 least about 86% amino acid sequence identity, more preferably at
least about 87% amino acid sequence identity, more preferably at
least about 88% amino acid sequence identity, more preferably at
least about 89% amino acid sequence identity, more preferably at
least about 90% amino acid sequence identity, more preferably at
10 least about 91% amino acid sequence identity, more preferably at
least about 92% amino acid sequence identity, more preferably at
least about 93% amino acid sequence identity, more preferably at
least about 94% amino acid sequence identity, more preferably at
least about 95% amino acid sequence identity, more preferably at
15 least about 96% amino acid sequence identity, more preferably at
least about 97% amino acid sequence identity, more preferably at
least about 98% amino acid sequence identity and yet more
preferably at least about 99% amino acid sequence identity with a
BR3 polypeptide encoded by a nucleic acid molecule shown in Figure
20 6 or a specified fragment thereof. BR3 variant polypeptides do not
encompass the native BR3 polypeptide sequence. Ordinarily, BR3
variant polypeptides are at least about 10 amino acids in length,
often at least about 20 amino acids in length, more often at least
about 30 amino acids in length, more often at least about 40 amino
25 acids in length, more often at least about 50 amino acids in
length, more often at least about 60 amino acids in length, more
often at least about 70 amino acids in length, more often at least
about 80 amino acids in length, more often at least about 90 amino
acids in length, more often at least about 100 amino acids in
30 length, more often at least about 150 amino acids in length, more
often at least about 200 amino acids in length, more often at least
about 250 amino acids in length, more often at least about 300
amino acids in length, or more.

The terms "TACI" or "TACI polypeptide" or "TACI receptor" when
35 used herein encompass "native sequence TACI polypeptides" and "TACI
variants" (which are further defined herein). "TACI" is a
designation given to those polypeptides which are encoded by the
nucleic acid molecules comprising the polynucleotide sequences

shown in Figure 1 and variants or fragments thereof, nucleic acid molecules comprising the sequence shown in the Figure 1 and variants thereof as well as fragments of the above. The TACI polypeptides of the invention may be isolated from a variety of
5 sources, such as from human tissue types or from another source, or prepared by recombinant and/or synthetic methods.

A "native sequence" TACI polypeptide comprises a polypeptide having the same amino acid sequence as the corresponding TACI polypeptide derived from nature. Such native sequence TACI
10 polypeptides can be isolated from nature or can be produced by recombinant and/or synthetic means. The term "native sequence TACI polypeptide" specifically encompasses naturally-occurring truncated or secreted forms (e.g., an extracellular domain sequence), naturally-occurring variant forms (e.g., alternatively spliced
15 forms) and naturally-occurring allelic variants of the polypeptide. The TACI polypeptides of the invention include but are not limited to the polypeptides described in von Bulow et al., supra and WO98/39361 published September 11, 1998, the spliced variant (referred to as "hTACI(265)" above and shown in Fig. 1C (SEQ ID
20 NO:17)), the TACI polypeptide comprising the contiguous sequence of amino acid residues 1-293 of Fig. 1 (SEQ ID NO:3), and the polypeptides disclosed in WO 00/40716 published July 13, 2000 and WO 01/85782 published November 15, 2001.

A TACI "extracellular domain" or "ECD" refers to a form of the
25 TACI polypeptide which is essentially free of the transmembrane and cytoplasmic domains. Ordinarily, a TACI polypeptide ECD will have less than about 1% of such transmembrane and/or cytoplasmic domains and preferably, will have less than about 0.5% of such domains. It will be understood that any transmembrane domain(s) identified for
30 the TACI polypeptides of the present invention are identified pursuant to criteria routinely employed in the art for identifying that type of hydrophobic domain. The exact boundaries of a transmembrane domain may vary but most likely by no more than about 5 amino acids at either end of the domain as initially identified.
35 ECD forms of TACI include those described in von Bulow et al., supra and WO98/39361.

"TACI variant" means a TACI polypeptide having at least about 80% amino acid sequence identity with the amino acid sequence of a

native sequence full length TACI or TACI ECD. Such TACI variant polypeptides include, for instance, TACI polypeptides wherein one or more amino acid residues are added, or deleted, at the N- and/or C-terminus, as well as within one or more internal domains, of the full-length amino acid sequence. Fragments of the TACI ECD are also contemplated. Ordinarily, a TACI variant polypeptide will have at least about 80% amino acid sequence identity, more preferably at least about 81% amino acid sequence identity, more preferably at least about 82% amino acid sequence identity, more preferably at least about 83% amino acid sequence identity, more preferably at least about 84% amino acid sequence identity, more preferably at least about 85% amino acid sequence identity, more preferably at least about 86% amino acid sequence identity, more preferably at least about 87% amino acid sequence identity, more preferably at least about 88% amino acid sequence identity, more preferably at least about 89% amino acid sequence identity, more preferably at least about 90% amino acid sequence identity, more preferably at least about 91% amino acid sequence identity, more preferably at least about 92% amino acid sequence identity, more preferably at least about 93% amino acid sequence identity, more preferably at least about 94% amino acid sequence identity, more preferably at least about 95% amino acid sequence identity, more preferably at least about 96% amino acid sequence identity, more preferably at least about 97% amino acid sequence identity, more preferably at least about 98% amino acid sequence identity and yet more preferably at least about 99% amino acid sequence identity with a TACI polypeptide encoded by a nucleic acid molecule shown in Figure 1 or a specified fragment thereof. TACI variant polypeptides do not encompass the native TACI polypeptide sequence. Ordinarily, TACI variant polypeptides are at least about 10 amino acids in length, often at least about 20 amino acids in length, more often at least about 30 amino acids in length, more often at least about 40 amino acids in length, more often at least about 50 amino acids in length, more often at least about 60 amino acids in length, more often at least about 70 amino acids in length, more often at least about 80 amino acids in length, more often at least about 90 amino acids in length, more often at least about 100 amino acids in length, more often at least about 150 amino acids in

length, more often at least about 200 amino acids in length, more often at least about 250 amino acids in length, more often at least about 300 amino acids in length, or more.

The term "TACIs" when used herein refers to polypeptides comprising the amino acid sequence of residues 1 to 246 of Figure 5B, or fragments or variants thereof, and which comprise a single cysteine rich domain. Optionally, such TACIs polypeptides comprise the contiguous sequence of residues 1 to 246 of Figure 5B. Optionally, such TACIs polypeptides are encoded by the nucleic acid molecules comprising the coding polynucleotide sequence shown in Figure 5A. The TACIs polypeptides of the invention may be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant and/or synthetic methods. A "native sequence" TACIs polypeptide comprises a polypeptide derived from nature. Such native sequence TACIs polypeptides can be isolated from nature or can be produced by recombinant and/or synthetic means. A TACIs polypeptide may comprise a fragment or variant of the polypeptide shown in Figure 5B and having at least about 80% amino acid sequence identity with the sequence shown in Figure 5B, more preferably at least about 81% amino acid sequence identity, more preferably at least about 82% amino acid sequence identity, more preferably at least about 83% amino acid sequence identity, more preferably at least about 84% amino acid sequence identity, more preferably at least about 85% amino acid sequence identity, more preferably at least about 86% amino acid sequence identity, more preferably at least about 87% amino acid sequence identity, more preferably at least about 88% amino acid sequence identity, more preferably at least about 89% amino acid sequence identity, more preferably at least about 90% amino acid sequence identity, more preferably at least about 91% amino acid sequence identity, more preferably at least about 92% amino acid sequence identity, more preferably at least about 93% amino acid sequence identity, more preferably at least about 94% amino acid sequence identity, more preferably at least about 95% amino acid sequence identity, more preferably at least about 96% amino acid sequence identity, more preferably at least about 97% amino acid sequence identity, more preferably at least about 98% amino acid sequence identity and yet more preferably at least about

99% amino acid sequence identity with a TACIs polypeptide encoded by an encoding nucleic acid sequence shown in Figure 5A or a specified fragment thereof. Such variant polypeptides include, for instance, polypeptides wherein one or more amino acid residues are added, or deleted, at the N- and/or C-terminus, as well as within one or more internal domains, of the amino acid sequence shown in Figure 5B.

A TACIs "extracellular domain" or "ECD" refers to a form of the TACIs polypeptide which is essentially free of the transmembrane and cytoplasmic domains. Ordinarily, a TACIs polypeptide ECD will have less than about 1% of such transmembrane and/or cytoplasmic domains and preferably, will have less than about 0.5% of such domains. It will be understood that any transmembrane domain(s) identified for the TACIs polypeptides of the present invention are identified pursuant to criteria routinely employed in the art for identifying that type of hydrophobic domain. The exact boundaries of a transmembrane domain may vary but most likely by no more than about 5 amino acids at either end of the domain as initially identified. ECD forms of TACIs include polypeptides comprising amino acid residues 1 to 119 of Figure 5B, and optionally a sequence of contiguous amino acid residues 1 to 119 of Figure 5B.

The terms "BCMA" or "BCMA polypeptide" or "BCMA receptor" when used herein encompass "native sequence BCMA polypeptides" and "BCMA variants" (which are further defined herein). "BCMA" is a designation given to those polypeptides which are encoded by the nucleic acid molecules comprising the polynucleotide sequences shown in Figure 2 and variants thereof, nucleic acid molecules comprising the sequence shown in the Figure 2 and variants thereof as well as fragments of the above. The BCMA polypeptides of the invention may be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant and/or synthetic methods.

A "native sequence" BCMA polypeptide comprises a polypeptide having the same amino acid sequence as the corresponding BCMA polypeptide derived from nature. Such native sequence BCMA polypeptides can be isolated from nature or can be produced by recombinant and/or synthetic means. The term "native sequence BCMA

polypeptide" specifically encompasses naturally-occurring truncated or secreted forms (e.g., an extracellular domain sequence), naturally-occurring variant forms (e.g., alternatively spliced forms) and naturally-occurring allelic variants of the polypeptide.

5 The BCMA polypeptides of the invention include the polypeptides described in Laabi et al., EMBO J., 11:3897-3904 (1992); Laabi et al., Nucleic Acids Res., 22:1147-1154 (1994); Gras et al., Int. Immunology, 7:1093-1106 (1995); Madry et al., Int. Immunology, 10:1693-1702 (1998); WO 00/50633 published November 16, 2000; WO
10 00/50633 published August 31, 2000; and the BCMA polypeptide comprising the contiguous sequence of amino acid residues 1-184 of Fig. 2 (SEQ ID NO:6).

A BCMA "extracellular domain" or "ECD" refers to a form of the BCMA polypeptide which is essentially free of the transmembrane and
15 cytoplasmic domains. Ordinarily, a BCMA polypeptide ECD will have less than about 1% of such transmembrane and/or cytoplasmic domains and preferably, will have less than about 0.5% of such domains. It will be understood that any transmembrane domain(s) identified for the BCMA polypeptides of the present invention are identified
20 pursuant to criteria routinely employed in the art for identifying that type of hydrophobic domain. The exact boundaries of a transmembrane domain may vary but most likely by no more than about 5 amino acids at either end of the domain as initially identified. ECD forms of BCMA include those described in Laabi et al., EMBO J.,
25 11:3897-3904 (1992); Laabi et al., Nucleic Acids Res., 22:1147-1154 (1994); Gras et al., Int. Immunology, 7:1093-1106 (1995); Madry et al., Int. Immunology, 10:1693-1702 (1998).

"BCMA variant" means a BCMA polypeptide having at least about 80% amino acid sequence identity with the amino acid sequence of a
30 native sequence BCMA or BCMA ECD. Such BCMA variant polypeptides include, for instance, BCMA polypeptides wherein one or more amino acid residues are added, or deleted, at the N- and/or C-terminus, as well as within one or more internal domains, of the full-length amino acid sequence. Fragments of the BCMA ECD are also
35 contemplated. Ordinarily, a BCMA variant polypeptide will have at least about 80% amino acid sequence identity, more preferably at least about 81% amino acid sequence identity, more preferably at least about 82% amino acid sequence identity, more preferably at

least about 83% amino acid sequence identity, more preferably at
least about 84% amino acid sequence identity, more preferably at
least about 85% amino acid sequence identity, more preferably at
least about 86% amino acid sequence identity, more preferably at
5 least about 87% amino acid sequence identity, more preferably at
least about 88% amino acid sequence identity, more preferably at
least about 89% amino acid sequence identity, more preferably at
least about 90% amino acid sequence identity, more preferably at
least about 91% amino acid sequence identity, more preferably at
10 least about 92% amino acid sequence identity, more preferably at
least about 93% amino acid sequence identity, more preferably at
least about 94% amino acid sequence identity, more preferably at
least about 95% amino acid sequence identity, more preferably at
least about 96% amino acid sequence identity, more preferably at
15 least about 97% amino acid sequence identity, more preferably at
least about 98% amino acid sequence identity and yet more
preferably at least about 99% amino acid sequence identity with a
BCMA polypeptide encoded by a nucleic acid molecule shown in Figure
2 or a specified fragment thereof. BCMA variant polypeptides do
20 not encompass the native BCMA polypeptide sequence. Ordinarily,
BCMA variant polypeptides are at least about 10 amino acids in
length, often at least about 20 amino acids in length, more often
at least about 30 amino acids in length, more often at least about
40 amino acids in length, more often at least about 50 amino acids
25 in length, more often at least about 60 amino acids in length, more
often at least about 70 amino acids in length, more often at least
about 80 amino acids in length, more often at least about 90 amino
acids in length, more often at least about 100 amino acids in
length, more often at least about 150 amino acids in length, more
30 often at least about 200 amino acids in length, more often at least
about 250 amino acids in length, more often at least about 300
amino acids in length, or more.

The terms "TALL-1" or "TALL-1 polypeptide" when used herein
encompass "native sequence TALL-1 polypeptides" and "TALL-1
35 variants". "TALL-1" is a designation given to those polypeptides
which are encoded by the nucleic acid molecules comprising the
polynucleotide sequences shown in Figure 3 and variants thereof,
nucleic acid molecules comprising the sequence shown in the Figure

3, and variants thereof as well as fragments of the above which have the biological activity of the native sequence TALL-1. Variants of TALL-1 will preferably have at least 80%, more preferably, at least 90%, and even more preferably, at least 95% amino acid sequence identity with the native sequence TALL-1 polypeptide shown in Figure 3. A "native sequence" TALL-1 polypeptide comprises a polypeptide having the same amino acid sequence as the corresponding TALL-1 polypeptide derived from nature. Such native sequence TALL-1 polypeptides can be isolated from nature or can be produced by recombinant and/or synthetic means. The term "native sequence TALL-1 polypeptide" specifically encompasses naturally-occurring truncated or secreted forms (e.g., an extracellular domain sequence), naturally-occurring variant forms (e.g., alternatively spliced forms) and naturally-occurring allelic variants of the polypeptide. The term "TALL-1" includes those polypeptides described in Shu et al., GenBank Accession No. AF136293; WO98/18921 published May 7, 1998; EP 869,180 published October 7, 1998; WO98/27114 published June 25, 1998; WO99/12964 published March 18, 1999; WO99/33980 published July 8, 1999; EP 869,180 published October 7, 1998; Moore et al., supra; Schneider et al., supra; and Mukhopadhyay et al., supra.

The terms "APRIL" or "APRIL polypeptide" when used herein encompass "native sequence APRIL polypeptides" and "APRIL variants". "APRIL" is a designation given to those polypeptides which are encoded by the nucleic acid molecules comprising the polynucleotide sequences shown in Figure 4A-4B and variants thereof, nucleic acid molecules comprising the sequence shown in the Figure 4A-4B, and variants thereof as well as fragments of the above which have the biological activity of the native sequence APRIL. Variants of APRIL will preferably have at least 80%, more preferably, at least 90%, and even more preferably, at least 95% amino acid sequence identity with the native sequence APRIL polypeptide shown in Figure 4A-4B. A "native sequence" APRIL polypeptide comprises a polypeptide having the same amino acid sequence as the corresponding APRIL polypeptide derived from nature. Such native sequence APRIL polypeptides can be isolated from nature or can be produced by recombinant and/or synthetic means. The term "native sequence APRIL polypeptide" specifically

encompasses naturally-occurring truncated or secreted forms (e.g., an extracellular domain sequence), naturally-occurring variant forms (e.g., alternatively spliced forms) and naturally-occurring allelic variants of the polypeptide. The term "APRIL" includes
5 those polypeptides described in Hahne et al., J. Exp. Med.,
188:1185-1190 (1998); GenBank Accession No. AF046888; WO 99/00518
published January 7, 1999; WO 99/35170 published July 15, 1999; WO
99/12965 published March 18, 1999; WO 99/33980 published July 8,
1999; WO 97/33902 published September 18, 1997; WO 99/11791
10 published March 11, 1999; EP 911,633 published March 28, 1999; and
WO99/50416 published October 7, 1999.

"Stringency" of hybridization reactions is readily determinable by one of ordinary skill in the art, and generally is an empirical calculation dependent upon probe length, washing
15 temperature, and salt concentration. In general, longer probes
require higher temperatures for proper annealing, while shorter
probes need lower temperatures. Hybridization generally depends on
the ability of denatured DNA to re-anneal when complementary
strands are present in an environment below their melting
20 temperature. The higher the degree of desired identity between the
probe and hybridizable sequence, the higher the relative
temperature which can be used. As a result, it follows that higher
relative temperatures would tend to make the reaction conditions
more stringent, while lower temperatures less so. For additional
25 details and explanation of stringency of hybridization reactions,
see Ausubel et al., Current Protocols in Molecular Biology, Wiley
Interscience Publishers, (1995).

"Stringent conditions" or "high stringency conditions", as defined herein, are identified by those that: (1) employ low ionic
30 strength and high temperature for washing, 0.015 M sodium
chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulfate at
50°C; (2) employ during hybridization a denaturing agent, 50% (v/v)
formamide with 0.1% bovine serum albumin/0.1% Ficoll/0.1%
polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 with
35 750 mM sodium chloride, 75 mM sodium citrate at 42°C; or (3) employ
50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50 mM
sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x
Denhardt's solution, sonicated salmon sperm DNA (50 µg/ml), 0.1%

SDS, and 10% dextran sulfate at 42°C, with washes at 42°C in 0.2 x SSC (sodium chloride/sodium citrate) and 50% formamide at 55°C, followed by a high-stringency wash consisting of 0.1 x SSC containing EDTA at 55°C.

- 5 "Moderately stringent conditions" are identified as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, New York: Cold Spring Harbor Press, 1989, and include the use of washing solution and hybridization conditions (e.g., temperature, ionic strength and %SDS) less stringent than those described above.
- 10 An example of moderately stringent conditions is overnight incubation at 37°C in a solution comprising: 20% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5 x Denhardt's solution, 10% dextran sulfate, and 20 mg/ml denatured sheared salmon sperm DNA, followed by washing the filters
- 15 in 1 x SSC at about 37-50°C. The skilled artisan will recognize how to adjust the temperature, ionic strength, etc. as necessary to accommodate factors such as probe length and the like.

Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For

20 example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is

25 operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by

30 ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

The terms "amino acid" and "amino acids" refer to all naturally occurring L-alpha-amino acids. This definition is meant

35 to include norleucine, ornithine, and homocysteine. The amino acids are identified by either the single-letter or three-letter designations:

	Asp	D	aspartic acid	Ile	I	isoleucine
	Thr	T	threonine	Leu	L	leucine
	Ser	S	serine	Tyr	Y	tyrosine
	Glu	E	glutamic acid	Phe	F	phenylalanine
5	Pro	P	proline	His	H	histidine
	Gly	G	glycine	Lys	K	lysine
	Ala	A	alanine	Arg	R	arginine
	Cys	C	cysteine	Trp	W	tryptophan
	Val	V	valine	Gln	Q	glutamine
10	Met	M	methionine	Asn	N	asparagine

In the Sequence Listing and Figures, certain other single-letter or three-letter designations may be employed to refer to and identify two or more amino acids or nucleotides at a given position in the sequence.

"Percent (%) amino acid sequence identity" with respect to the ligand or receptor polypeptide sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in such a ligand or receptor sequence identified herein, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are obtained as described below by using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc. and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available through

Genentech, Inc., South San Francisco, California. The ALIGN-2 program should be compiled for use on a UNIX operating system, preferably digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

5 As used herein, the term "immunoadhesin" designates antibody-like molecules which combine the binding specificity of a heterologous protein (an "adhesin") with the effector functions of immunoglobulin constant domains. Structurally, the immunoadhesins
10 comprise a fusion of an amino acid sequence with the desired binding specificity which is other than the antigen recognition and binding site of an antibody (i.e., is "heterologous"), and an immunoglobulin constant domain sequence. The adhesin part of an immunoadhesin molecule typically is a contiguous amino acid
15 sequence comprising at least the binding site of a receptor or a ligand. The immunoglobulin constant domain sequence in the immunoadhesin may be obtained from any immunoglobulin, such as IgG-1, IgG-2, IgG-3, or IgG-4 subtypes, IgA (including IgA-1 and IgA-2), IgE, IgD or IgM.

The term "antagonist" is used in the broadest sense, and
20 includes any molecule that partially or fully blocks, inhibits, or neutralizes one or more biological activities of TALL-1 polypeptide, APRIL polypeptide, or both TALL-1 and APRIL, *in vitro*, *in situ*, or *in vivo*. Examples of such biological activities of TALL-1 and APRIL polypeptides include binding of TALL-1 or APRIL to
25 TACI, BCMA, TACIs or BR3, activation of NF-KB and activation of proliferation and of Ig secretion by B cells, immune-related conditions such as rheumatoid arthritis and lupus, as well as those further reported in the literature. An antagonist may function in a direct or indirect manner. For instance, the antagonist may
30 function to partially or fully block, inhibit or neutralize one or more biological activities of TALL-1 polypeptide, APRIL polypeptide, or both TALL-1 and APRIL, *in vitro*, *in situ*, or *in vivo* as a result of its direct binding to TACIs or TACI. The antagonist may also function indirectly to partially or fully
35 block, inhibit or neutralize one or more biological activities of TALL-1 polypeptide, APRIL polypeptide, or both TALL-1 and APRIL, *in vitro*, *in situ*, or *in vivo* as a result of, e.g., blocking or inhibiting its binding to BCMA or BR3, or another effector

molecule. The antagonist molecule may comprise a "dual" antagonist activity wherein the molecule is capable of partially or fully blocking, inhibiting or neutralizing a biological activity of both TALL-1 and APRIL.

5 The term "agonist" is used in the broadest sense, and includes any molecule that partially or fully enhances, stimulates or activates one or more biological activities of TACI or TACIs polypeptide, or both TACIs and TACI, *in vitro*, *in situ*, or *in vivo*. Examples of such biological activities of TACIs and TACI may
10 include activation of NF-KB, induction or inhibition of immunoglobulin production and secretion, and cell proliferation. An agonist may function in a direct or indirect manner. For instance, the agonist may function to partially or fully enhance, stimulate or activate one or more biological activities of TACIs
15 polypeptide, TACI polypeptide, or both TACIs and TACI, *in vitro*, *in situ*, or *in vivo* as a result of its direct binding to TACIs or TACI, which may cause receptor activation or signal transduction. The agonist may also function indirectly to partially or fully enhance, stimulate or activate one or more biological activities of
20 TACIs polypeptide, TACI polypeptide, or both TACIs and TACI, *in vitro*, *in situ*, or *in vivo* as a result of, e.g., stimulating another effector molecule which then causes TACIs or TACI receptor activation or signal transduction.

 The term "antibody" is used in the broadest sense and
25 specifically covers, for example, single monoclonal antibodies against BR3, TACIs, TALL-1, APRIL, TACI, or BCMA, antibody compositions with polyeptopic specificity, single chain antibodies, and fragments of antibodies. "Antibody" as used herein includes intact immunoglobulin or antibody molecules, polyclonal
30 antibodies, multispecific antibodies (i.e., bispecific antibodies formed from at least two intact antibodies) and immunoglobulin fragments (such as Fab, F(ab')₂, or Fv), so long as they exhibit any of the desired agonistic or antagonistic properties described herein.

35 Antibodies are typically proteins or polypeptides which exhibit binding specificity to a specific antigen. Native antibodies are usually heterotetrameric glycoproteins, composed of two identical

light (L) chains and two identical heavy (H) chains. Typically, each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies between the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light and heavy chain variable domains [Chothia et al., J. Mol. Biol., 186:651-663 (1985); Novotny and Haber, Proc. Natl. Acad. Sci. USA, 82:4592-4596 (1985)]. The light chains of antibodies from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG-1, IgG-2, IgG-3, and IgG-4; IgA-1 and IgA-2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively.

"Antibody fragments" comprise a portion of an intact antibody, generally the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments, diabodies, single chain antibody molecules, and multispecific antibodies formed from antibody fragments.

The term "variable" is used herein to describe certain portions of the variable domains which differ in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not usually evenly distributed through the variable domains of antibodies. It is typically concentrated in three segments called

complementarity determining regions (CDRs) or hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of the variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a β -sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β -sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen binding site of antibodies [see Kabat, E.A. et al., Sequences of Proteins of Immunological Interest, National Institutes of Health, Bethesda, MD (1987)]. The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen.

The monoclonal antibodies herein include chimeric, hybrid and recombinant antibodies produced by splicing a variable (including hypervariable) domain of the antibody of interest with a constant domain (e.g. "humanized" antibodies), or a light chain with a heavy chain, or a chain from one species with a chain from another species, or fusions with heterologous proteins, regardless of species of origin or immunoglobulin class or subclass designation, as well as antibody fragments (e.g., Fab, F(ab')₂, and Fv), so long as they exhibit the desired biological activity or properties. See, e.g. U.S. Pat. No. 4,816,567 and Mage et al., in Monoclonal Antibody Production Techniques and Applications, pp.79-97 (Marcel Dekker,

Inc.: New York, 1987).

Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring
5 production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler and Milstein, Nature, 256:495 (1975), or may be made by recombinant DNA methods such as described in U.S. Pat. No.
10 4,816,567. The "monoclonal antibodies" may also be isolated from phage libraries generated using the techniques described in McCafferty et al., Nature, 348:552-554 (1990), for example.

"Humanized" forms of non-human (e.g. murine) antibodies are specific chimeric immunoglobulins, immunoglobulin chains, or
15 fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the
20 recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, the
25 humanized antibody may comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two,
30 variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant
35 region or domain (Fc), typically that of a human immunoglobulin.

A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a

human and/or has been made using any of the techniques for making human antibodies known in the art or as disclosed herein. This definition of a human antibody includes antibodies comprising at least one human heavy chain polypeptide or at least one human light chain polypeptide, for example an antibody comprising murine light chain and human heavy chain polypeptides. Human antibodies can be produced using various techniques known in the art. In one embodiment, the human antibody is selected from a phage library, where that phage library expresses human antibodies (Vaughan et al. Nature Biotechnology, 14:309-314 (1996); Sheets et al. PNAS, (USA) 95:6157-6162 (1998)); Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991)). Human antibodies can also be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks et al., Bio/Technology, 10: 779-783 (1992); Lonberg et al., Nature, 368: 856-859 (1994); Morrison, Nature, 368:812-13 (1994); Fishwild et al., Nature Biotechnology, 14: 845-51 (1996); Neuberger, Nature Biotechnology, 14: 826 (1996); Lonberg and Huszar, Intern. Rev. Immunol., 13:65-93 (1995). Alternatively, the human antibody may be prepared via immortalization of human B lymphocytes producing an antibody directed against a target antigen (such B lymphocytes may be recovered from an individual or may have been immunized in vitro). See, e.g., Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985); Boerner et al., J. Immunol., 147 (1):86-95 (1991); and US Pat No. 5,750,373.

The term "Fc region" is used to define the C-terminal region of an immunoglobulin heavy chain which may be generated by papain digestion of an intact antibody. The Fc region may be a native sequence Fc region or a variant Fc region. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the

human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at about position Cys226, or from about position Pro230, to the carboxyl-terminus of the Fc region (using herein the numbering system according to Kabat et al., supra). The Fc region of an immunoglobulin generally comprises two constant domains, a CH2 domain and a CH3 domain, and optionally comprises a CH4 domain.

By "Fc region chain" herein is meant one of the two polypeptide chains of an Fc region.

The "CH2 domain" of a human IgG Fc region (also referred to as "Cγ2" domain) usually extends from an amino acid residue at about position 231 to an amino acid residue at about position 340. The CH2 domain is unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains are interposed between the two CH2 domains of an intact native IgG molecule. It has been speculated that the carbohydrate may provide a substitute for the domain-domain pairing and help stabilize the CH2 domain. Burton, *Molec. Immunol.* 22:161-206 (1985). The CH2 domain herein may be a native sequence CH2 domain or variant CH2 domain.

The "CH3 domain" comprises the stretch of residues C-terminal to a CH2 domain in an Fc region (*i.e.* from an amino acid residue at about position 341 to an amino acid residue at about position 447 of an IgG). The CH3 region herein may be a native sequence CH3 domain or a variant CH3 domain (e.g. a CH3 domain with an introduced "protuberance" in one chain thereof and a corresponding introduced "cavity" in the other chain thereof; see US Patent No. 5,821,333). Such variant CH3 domains may be used to make multispecific (e.g. bispecific) antibodies as herein described.

"Hinge region" is generally defined as stretching from about Glu216, or about Cys226, to about Pro230 of human IgG1' (Burton, *Molec. Immunol.* 22:161-206 (1985)). Hinge regions of other IgG isotypes may be aligned with the IgG1 sequence by placing the first and last cysteine residues forming inter-heavy chain S-S bonds in the same positions. The hinge region herein may be a native sequence hinge region or a variant hinge region. The two polypeptide chains of a variant hinge region generally retain at

least one cysteine residue per polypeptide chain, so that the two polypeptide chains of the variant hinge region can form a disulfide bond between the two chains. The preferred hinge region herein is a native sequence human hinge region, e.g. a native sequence human IgG1 hinge region.

A "functional Fc region" possesses at least one "effector function" of a native sequence Fc region. Exemplary "effector functions" include Clq binding; complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor; BCR), etc. Such effector functions generally require the Fc region to be combined with a binding domain (e.g. an antibody variable domain) and can be assessed using various assays known in the art for evaluating such antibody effector functions.

A "native sequence Fc region" comprises an amino acid sequence identical to the amino acid sequence of a Fc region found in nature. A "variant Fc region" comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification. Preferably, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, e.g. from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region herein will preferably possess at least about 80% sequence identity with a native sequence Fc region and/or with an Fc region of a parent polypeptide, and most preferably at least about 90% sequence identity therewith, more preferably at least about 95% sequence identity therewith.

"Antibody-dependent cell-mediated cytotoxicity" and "ADCC" refer to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors (FCRs) (e.g. Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cell. The primary cells for mediating ADCC, NK cells, express FcγRIII only,

whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, Annu. Rev. Immunol., 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an *in vitro* ADCC assay, such as that described in US Patent No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in a animal model such as that disclosed in Clynes et al. PNAS (USA), 95:652-656 (1998).

"Human effector cells" are leukocytes which express one or more FcRs and perform effector functions. Preferably, the cells express at least FcγRIII and perform ADCC effector function. Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils; with PBMCs and NK cells being preferred. The effector cells may be isolated from a native source thereof, e.g. from blood or PBMCs as described herein.

The terms "Fc receptor" and "FcR" are used to describe a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcγRII receptors include FcγRIIA (an "activating receptor") and FcγRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain (reviewed in Daëron, Annu. Rev. Immunol., 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, Annu. Rev. Immunol., 9:457-92 (1991); Capel et al., Immunomethods, 4:25-

34 (1994); and de Haas et al., J. Lab. Clin. Med., 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., J. Immunol., 117:587 (1976); and Kim et al., J. Immunol., 24:249 (1994)).

"Complement dependent cytotoxicity" and "CDC" refer to the lysing of a target in the presence of complement. The complement activation pathway is initiated by the binding of the first component of the complement system (C1q) to a molecule (e.g. an antibody) complexed with a cognate antigen. To assess complement activation, a CDC assay, e.g. as described in Gazzano-Santoro et al., J. Immunol. Methods, 202:163 (1996), may be performed.

An "affinity matured" antibody is one with one or more alterations in one or more CDRs thereof which result an improvement in the affinity of the antibody for antigen, compared to a parent antibody which does not possess those alteration(s). Preferred affinity matured antibodies will have nanomolar or even picomolar affinities for the target antigen. Affinity matured antibodies are produced by procedures known in the art. Marks et al. Bio/Technology, 10:779-783 (1992) describes affinity maturation by VH and VL domain shuffling. Random mutagenesis of CDR and/or framework residues is described by: Barbas et al. Proc Nat. Acad. Sci, USA 91:3809-3813 (1994); Schier et al. Gene, 169:147-155 (1995); Yelton et al. J. Immunol., 155:1994-2004 (1995); Jackson et al., J. Immunol., 154(7):3310-9 (1995); and Hawkins et al, J. Mol. Biol., 226:889-896 (1992).

The term "immunospecific" as used in "immunospecific binding of antibodies" for example, refers to the antigen specific binding interaction that occurs between the antigen-combining site of an antibody and the specific antigen recognized by that antibody.

"Isolated," when used to describe the various proteins disclosed herein, means protein that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the protein, and may include enzymes, hormones,

and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the protein will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated protein includes protein *in situ* within recombinant cells, since at least one component of the protein natural environment will not be present. Ordinarily, however, isolated protein will be prepared by at least one purification step.

"Treatment" or "therapy" refer to both therapeutic treatment and prophylactic or preventative measures.

"Mammal" for purposes of treatment or therapy refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc. Preferably, the mammal is human.

"TALL-1 -related pathological condition" and "APRIL-related pathological condition" refer to pathologies or conditions associated with abnormal levels of expression or activity of TALL-1 or APRIL, respectively, in excess of, or less than, levels of expression or activity in normal healthy mammals, where such excess or diminished levels occur in a systemic, localized, or particular tissue or cell type or location in the body. TALL-1 -related pathological conditions and APRIL-related pathological conditions include acute and chronic immune related diseases and cancer.

The terms "cancer", "cancerous", and "malignant" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, carcinoma including adenocarcinoma, lymphoma, blastoma, melanoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, gastrointestinal cancer, Hodgkin's and non-Hodgkin's lymphoma, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer such as hepatic carcinoma and hepatoma, bladder cancer, breast cancer, colon cancer, colorectal cancer, endometrial carcinoma, myeloma (such as multiple myeloma), salivary gland carcinoma, kidney cancer such as renal cell carcinoma and Wilms'

tumors, basal cell carcinoma, melanoma, prostate cancer, vulval cancer, thyroid cancer, testicular cancer, esophageal cancer, and various types of head and neck cancer. Optional cancers for treatment herein include lymphoma, leukemia and myeloma, and
5 subtypes thereof, such as Burkitt's lymphoma, multiple myeloma, acute lymphoblastic or lymphocytic leukemia, non-Hodgkin's and Hodgkin's lymphoma, and acute myeloid leukemia.

The term "immune related disease" means a disease in which a component of the immune system of a mammal causes, mediates or
10 otherwise contributes to a morbidity in the mammal. Also included are diseases in which stimulation or intervention of the immune response has an ameliorative effect on progression of the disease. Included within this term are autoimmune diseases, immune-mediated inflammatory diseases, non-immune-mediated inflammatory diseases,
15 infectious diseases, and immunodeficiency diseases. Examples of immune-related and inflammatory diseases, some of which are immune or T cell mediated, which can be treated according to the invention include systemic lupus erythematosus, rheumatoid arthritis, juvenile chronic arthritis, spondyloarthropathies, systemic
20 sclerosis (scleroderma), idiopathic inflammatory myopathies (dermatomyositis, polymyositis), Sjogren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia (immune pancytopenia, paroxysmal nocturnal hemoglobinuria), autoimmune thrombocytopenia (idiopathic thrombocytopenic purpura, immune-
25 mediated thrombocytopenia), thyroiditis (Grave's disease, Hashimoto's thyroiditis, juvenile lymphocytic thyroiditis, atrophic thyroiditis), diabetes mellitus, immune-mediated renal disease (glomerulonephritis, tubulointerstitial nephritis), demyelinating diseases of the central and peripheral nervous systems such as
30 multiple sclerosis, idiopathic demyelinating polyneuropathy or Guillain-Barré syndrome, and chronic inflammatory demyelinating polyneuropathy, hepatobiliary diseases such as infectious hepatitis (hepatitis A, B, C, D, E and other non-hepatotropic viruses), autoimmune chronic active hepatitis, primary biliary cirrhosis,
35 granulomatous hepatitis, and sclerosing cholangitis, inflammatory and fibrotic lung diseases such as inflammatory bowel disease (ulcerative colitis: Crohn's disease), gluten-sensitive enteropathy, and Whipple's disease, autoimmune or immune-mediated

skin diseases including bullous skin diseases, erythema multiforme and contact dermatitis, psoriasis, allergic diseases such as asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity and urticaria, immunologic diseases of the lung such as
5 eosinophilic pneumonias, idiopathic pulmonary fibrosis and hypersensitivity pneumonitis, transplantation associated diseases including graft rejection and graft-versus-host-disease. Infectious diseases include AIDS (HIV infection), hepatitis A, B, C, D, and E, bacterial infections, fungal infections, protozoal
10 infections and parasitic infections.

"Autoimmune disease" is used herein in a broad, general sense to refer to disorders or conditions in mammals in which destruction of normal or healthy tissue arises from humoral or cellular immune responses of the individual mammal to his or her own tissue
15 constituents. Examples include, but are not limited to, lupus erythematosus, thyroiditis, rheumatoid arthritis, psoriasis, multiple sclerosis, autoimmune diabetes, and inflammatory bowel disease (IBD).

The term "prodrug" as used in this application refers to a
20 precursor or derivative form of a pharmaceutically active substance that is less cytotoxic to cancer cells compared to the parent drug and is capable of being enzymatically activated or converted into the more active parent form. See, e.g., Wilman, "Prodrugs in Cancer Chemotherapy" *Biochemical Society Transactions*, 14, pp. 375-
25 382, 615th Meeting Belfast (1986) and Stella et al., "Prodrugs: A Chemical Approach to Targeted Drug Delivery," *Directed Drug Delivery*, Borchardt et al., (ed.), pp. 247-267, Humana Press (1985). The prodrugs of this invention include, but are not limited to, phosphate-containing prodrugs, thiophosphate-containing
30 prodrugs, sulfate-containing prodrugs, peptide-containing prodrugs, D-amino acid-modified prodrugs, glycosylated prodrugs, beta-lactam-containing prodrugs, optionally substituted phenoxyacetamide-containing prodrugs or optionally substituted phenylacetamide-containing prodrugs, 5-fluorocytosine and other 5-fluorouridine
35 prodrugs which can be converted into the more active cytotoxic free drug. Examples of cytotoxic drugs that can be derivatized into a prodrug form for use in this invention include, but are not limited to, those chemotherapeutic agents described below.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g. At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³² and radioactive isotopes of Lu), chemotherapeutic agents, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of conditions like cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide (CYTOXAN™); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CBI-TMI); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, ranimustine; antibiotics such as the enediyne antibiotics (e.g. calicheamicin, especially calicheamicin γ_1^I and calicheamicin θ_1^I , see, e.g., Agnew Chem Intl. Ed. Engl., 33:183-186 (1994); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabycin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin

(including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine, 5-FU; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiothane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK®; razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2, 2', 2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g. paclitaxel (TAXOL®, Bristol-Myers Squibb Oncology, Princeton, NJ) and doxetaxel (TAXOTERE®, Rhône-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine; 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as cisplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitomycin C; mitoxantrone; vincristine; vinorelbine; navelbine; novantrone; teniposide; daunomycin; aminopterin; xeloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoic acid; capecitabine; and pharmaceutically acceptable salts, acids or derivatives of any of the above. Also included in this

definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens including for example tamoxifen, raloxifene, aromatase inhibiting 4(5)-imidazoles, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and toremifene (Fareston); and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell, either in vitro or in vivo. Thus, the growth inhibitory agent is one which significantly reduces the percentage of cells overexpressing such genes in S phase. Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxol, and topo II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in *The Molecular Basis of Cancer*, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle regulation, oncogens, and antineoplastic drugs" by Murakami et al. (WB Saunders: Philadelphia, 1995), especially p. 13.

The term "cytokine" is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor- α and - β ; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin;

vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors; platelet-growth factor; transforming growth factors (TGFs) such as TGF- α and TGF- β ; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors;

5 interferons such as interferon- α , - β , and -gamma; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12; and other polypeptide factors

10 including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

15 II. Methods and Materials

The invention provides methods and materials for modulating TALL-1, APRIL, TACI, BCMA, TACIs, and/or BR3 activity in mammalian cells which comprise exposing the cells to a desired amount of TACI antibody. Preferably, the amount of TACI antibody employed will be

20 an amount effective to affect the binding and/or activity of the respective ligand or respective receptor to achieve a therapeutic effect. This can be accomplished in vivo or ex vivo in accordance, for instance, with the methods described below and in the Examples. Exemplary conditions or disorders to be treated with such TACI

25 antibodies include conditions in mammals clinically referred to as autoimmune diseases, including but not limited to rheumatoid arthritis, multiple sclerosis, psoriasis, and lupus or other pathological conditions in which B cell response(s) in mammals is abnormally upregulated such as cancer.

30 A. ANTIBODIES

Anti-TACI receptor antibodies are provided herein and may be employed in the presently disclosed methods. Monoclonal antibodies may be prepared using hybridoma methods, such as those described by Kohler and Milstein, Nature, 256:495 (1975). In a hybridoma method,

35 a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will

specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*.

The immunizing agent will typically include a TACI polypeptide (or a TACI ECD) or a fusion protein thereof, such as a TACI ECD-IgG fusion protein. The immunizing agent may alternatively comprise a
5 fragment or portion of TACI having one or more amino acids that participate in the binding of TALL-1 or APRIL to TACI. In a preferred embodiment, the immunizing agent comprises an extracellular domain sequence of TACI.

10 Generally, either peripheral blood lymphocytes ("PBLs") are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a
15 hybridoma cell [Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986) pp. 59-103]. Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be
20 cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically
25 will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the
30 selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human
35 myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies [Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New

York, (1987) pp. 51-63].

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against TACI. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, Anal. Biochem., 107:220 (1980).

After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods [Goding, supra]. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium or RPMI-1640 medium. Alternatively, the hybridoma cells may be grown *in vivo* as ascites in a mammal.

The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences, Morrison, et al., Proc. Nat. Acad. Sci. 81, 6851 (1984), or by

covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody of the invention, or they are substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for TACI and another antigen-combining site having specificity for a different antigen.

Chimeric or hybrid antibodies also may be prepared in vitro using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

Single chain Fv fragments may also be produced, such as described in Iliades et al., FEBS Letters, 409:437-441 (1997). Coupling of such single chain fragments using various linkers is described in Kortt et al., Protein Engineering, 10:423-433 (1997).

A variety of techniques for the recombinant production and manipulation of antibodies are well known in the art. Illustrative examples of such techniques that are typically utilized by skilled artisans are described in greater detail below.

(i) *Humanized antibodies*

Generally, a humanized antibody has one or more amino acid residues introduced into it from a non-human source. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody.

Accordingly, such "humanized" antibodies are chimeric antibodies wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically

human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

It is important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three dimensional models of the parental and humanized sequences. Three dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e. the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequence so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

(ii) Human antibodies

Human monoclonal antibodies can be made by the hybridoma method. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described, for example, by Kozbor, J. Immunol. 133, 3001 (1984), and Brodeur, et al., Monoclonal Antibody Production Techniques and Applications, pp.51-63 (Marcel Dekker, Inc., New York, 1987).

It is now possible to produce transgenic animals (e.g. mice) that are capable, upon immunization, of producing a repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge.

See, e.g. Jakobovits et al., Proc. Natl. Acad. Sci. USA **90**, 2551-255 (1993); Jakobovits et al., Nature **362**, 255-258 (1993).

Mendez et al. (*Nature Genetics* **15**: 146-156 [1997]) have further improved the technology and have generated a line of transgenic mice designated as "Xenomouse II" that, when challenged with an antigen, generates high affinity fully human antibodies. This was achieved by germ-line integration of megabase human heavy chain and light chain loci into mice with deletion into endogenous J_H segment as described above. The Xenomouse II harbors 1,020 kb of human heavy chain locus containing approximately 66 V_H genes, complete D_H and J_H regions and three different constant regions (μ , δ and γ), and also harbors 800 kb of human κ locus containing 32 V κ genes, J κ segments and C κ genes. The antibodies produced in these mice closely resemble that seen in humans in all respects, including gene rearrangement, assembly, and repertoire. The human antibodies are preferentially expressed over endogenous antibodies due to deletion in endogenous J_H segment that prevents gene rearrangement in the murine locus.

Alternatively, the phage display technology (McCafferty et al., Nature **348**, 552-553 [1990]) can be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats; for their review see, e.g. Johnson, Kevin S. and Chiswell, David J., Current Opinion in Structural Biology **3**, 564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson et al., Nature **352**, 624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random

combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks et al., J. Mol. Biol. 222, 581-597 (1991), or Griffith et al., EMBO J. 12, 725-734 (1993). In a natural immune response, antibody genes accumulate mutations at a high rate (somatic hypermutation). Some of the changes introduced will confer higher affinity, and B cells displaying high-affinity surface immunoglobulin are preferentially replicated and differentiated during subsequent antigen challenge. This natural process can be mimicked by employing the technique known as "chain shuffling" (Marks et al., Bio/Technol. 10, 779-783 [1992]). In this method, the affinity of "primary" human antibodies obtained by phage display can be improved by sequentially replacing the heavy and light chain V region genes with repertoires of naturally occurring variants (repertoires) of V domain genes obtained from unimmunized donors. This technique allows the production of antibodies and antibody fragments with affinities in the nM range. A strategy for making very large phage antibody repertoires (also known as "the mother-of-all libraries") has been described by Waterhouse et al., Nucl. Acids Res. 21, 2265-2266 (1993). Gene shuffling can also be used to derive human antibodies from rodent antibodies, where the human antibody has similar affinities and specificities to the starting rodent antibody. According to this method, which is also referred to as "epitope imprinting", the heavy or light chain V domain gene of rodent antibodies obtained by phage display technique is replaced with a repertoire of human V domain genes, creating rodent-human chimeras. Selection on antigen results in isolation of human variable capable of restoring a functional antigen-binding site, i.e. the epitope governs (imprints) the choice of partner. When the process is repeated in order to replace the remaining rodent V domain, a human antibody is obtained (see PCT patent application WO 93/06213, published 1 April 1993). Unlike traditional humanization of rodent antibodies by CDR grafting, this technique provides completely human antibodies, which have no framework or CDR residues of rodent origin.

As discussed below, the antibodies of the invention may optionally comprise monomeric, antibodies, dimeric antibodies, as well as multivalent forms of antibodies. Those skilled in the art may construct such dimers or multivalent forms by techniques known in the art. Methods for preparing monovalent antibodies are also well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues are substituted with another amino acid residue or are deleted so as to prevent crosslinking.

(iii) *Bispecific antibodies*

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for the TACIs or BR3 receptor, the other one is for any other antigen such as BCMA or BR3 receptor, and preferably for another receptor or receptor subunit. For example, bispecific antibodies specifically binding a TACI receptor and another apoptosis-signalling receptor are within the scope of the present invention.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two heavy chains have different specificities (Millstein and Cuello, *Nature* 305, 537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in PCT application publication No. WO 93/08829 (published 13 May 1993), and in Traunecker et al., *EMBO* 10, 3655-3659 (1991).

According to a different and more preferred approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin

heavy chain constant domain, comprising at least part of the hinge, CH2 and CH3 regions. It is preferred to have the first heavy chain constant region (CH1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are cotransfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance. In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in PCT Publication No. WO 94/04690, published on March 3, 1994.

For further details of generating bispecific antibodies see, for example, Suresh et al., Methods in Enzymology 121, 210 (1986).

(iv) *Heteroconjugate antibodies*

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (PCT application publication Nos. WO 91/00360 and WO 92/200373; EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Patent No. 4,676,980, along with a number of cross-linking techniques.

(v) *Antibody fragments*

In certain embodiments, the anti-TACI antibody (including murine, human and humanized antibodies, and antibody variants) is an antibody fragment. Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al., *J. Biochem. Biophys. Methods* 24:107-117 (1992) and Brennan et al., *Science* 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. For example, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form F(ab')₂ fragments (Carter et al., *Bio/Technology* 10:163-167 (1992)). In another embodiment, the F(ab')₂ is formed using the leucine zipper GCN4 to promote assembly of the F(ab')₂ molecule. According to another approach, Fv, Fab or F(ab')₂ fragments can be isolated directly from recombinant host cell culture. A variety of techniques for the production of antibody fragments will be apparent to the skilled practitioner. For instance, digestion can be performed using papain. Examples of papain digestion are described in WO 94/29348 published 12/22/94 and U.S. Patent No. 4,342,566. Papain digestion of antibodies typically produces two identical antigen binding fragments, called Fab fragments, each with a single antigen binding site, and a residual Fc fragment. Pepsin treatment yields an F(ab')₂ fragment that has two antigen combining sites and is still capable of cross-linking antigen.

The Fab fragments produced in the antibody digestion also contain the constant domains of the light chain and the first constant domain (CH₁) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH₁ domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')₂ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

Antibodies are glycosylated at conserved positions in their constant regions (Jefferis and Lund, Chem. Immunol. 65:111-128 [1997]; Wright and Morrison, TibTECH 15:26-32 [1997]). The oligosaccharide side chains of the immunoglobulins affect the protein's function (Boyd et al., Mol. Immunol. 32:1311-1318 [1996]; Wittwe and Howard, Biochem. 29:4175-4180 [1990]), and the intramolecular interaction between portions of the glycoprotein which can affect the conformation and presented three-dimensional surface of the glycoprotein (Hefferis and Lund, *supra*; Wyss and Wagner, Current Opin. Biotech. 7:409-416 [1996]).

Oligosaccharides may also serve to target a given glycoprotein to certain molecules based upon specific recognition structures. For example, it has been reported that in agalactosylated IgG, the oligosaccharide moiety 'flips' out of the inter-CH2 space and terminal N-acetylglucosamine residues become available to bind mannose binding protein (Malhotra et al., Nature Med. 1:237-243 [1995]). Removal by glycopeptidase of the oligosaccharides from CAMPATH-1H (a recombinant humanized murine monoclonal IgG1 antibody which recognizes the CDw52 antigen of human lymphocytes) produced in Chinese Hamster Ovary (CHO) cells resulted in a complete reduction in complement mediated lysis (CMCL) (Boyd et al., Mol. Immunol. 32:1311-1318 [1996]), while selective removal of sialic acid residues using neuraminidase resulted in no loss of DMCL. Glycosylation of antibodies has also been reported to affect antibody-dependent cellular cytotoxicity (ADCC). In particular, CHO cells with tetracycline-regulated expression of $\beta(1,4)$ -N-acetylglucosaminyltransferase III (GnTIII), a glycosyltransferase catalyzing formation of bisecting GlcNAc, was reported to have improved ADCC activity (Umana et al., Mature Biotech. 17:176-180 [1999]).

Glycosylation variants of antibodies are variants in which the glycosylation pattern of an antibody is altered. By altering is meant deleting one or more carbohydrate moieties found in the antibody, adding one or more carbohydrate moieties to the antibody, changing the composition of glycosylation (glycosylation pattern), the extent of glycosylation, etc. Glycosylation variants may, for example, be prepared by removing, changing

and/or adding one or more glycosylation sites in the nucleic acid sequence encoding the antibody.

Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

The glycosylation (including glycosylation pattern) of antibodies may also be altered without altering the underlying nucleotide sequence. Glycosylation largely depends on the host cell used to express the antibody. Since the cell type used for expression of recombinant glycoproteins, e.g. antibodies, as potential therapeutics is rarely the native cell, significant variations in the glycosylation pattern of the antibodies can be expected (see, e.g. Hse et al., J. Biol. Chem. 272:9062-9070 [1997]). In addition to the choice of host cells, factors which affect glycosylation during recombinant production of antibodies include growth mode, media formulation, culture density, oxygenation, pH, purification schemes and the like. Various methods have been proposed to alter the glycosylation pattern achieved in a particular host organism including introducing or overexpressing certain enzymes involved in oligosaccharide production (U. S. Patent Nos. 5,047,335; 5,510,261 and 5,278,299).

Glycosylation, or certain types of glycosylation, can be enzymatically removed from the glycoprotein, for example using endoglycosidase H (Endo H). In addition, the recombinant host cell can be genetically engineered, e.g. make defective in processing certain types of polysaccharides. These and similar techniques are well known in the art.

The glycosylation structure of antibodies can be readily analyzed by conventional techniques of carbohydrate analysis, including lectin chromatography, NMR, Mass spectrometry, HPLC, GPC, monosaccharide compositional analysis, sequential enzymatic digestion, and HPAEC-PAD, which uses high pH anion exchange chromatography to separate oligosaccharides based on charge. Methods for releasing oligosaccharides for analytical purposes are also known, and include, without limitation, enzymatic treatment (commonly performed using peptide-N-glycosidase F/endo- β -galactosidase), elimination using harsh alkaline environment to release mainly O-linked structures, and chemical methods using anhydrous hydrazine to release both N- and O-linked oligosaccharides.

Triabodies are also within the scope of the invention. Such antibodies are described for instance in Iliades et al., supra and Kortt et al., supra.

The antibodies of the present invention may be modified by conjugating the antibody to a cytotoxic agent (like a toxin molecule) or a prodrug-activating enzyme which converts a prodrug (e.g. a peptidyl chemotherapeutic agent, see WO81/01145) to an active anti-cancer drug. See, for example, WO 88/07378 and U.S. Patent No. 4,975,278. This technology is also referred to as "Antibody Dependent Enzyme Mediated Prodrug Therapy" (ADEPT).

The enzyme component of the immunoconjugate useful for ADEPT includes any enzyme capable of acting on a prodrug in such a way so as to convert it into its more active, cytotoxic form. Enzymes that are useful in the method of this invention include, but are not limited to, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia

protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; caspases such as caspase-3; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as beta-galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs; beta-lactamase useful for converting drugs derivatized with beta-lactams into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as "abzymes", can be used to convert the prodrugs of the invention into free active drugs (see, e.g., Massey, *Nature* 328: 457-458 (1987)). Antibody-abzyme conjugates can be prepared as described herein for delivery of the abzyme to a tumor cell population.

The enzymes can be covalently bound to the antibodies by techniques well known in the art such as the use of heterobifunctional crosslinking reagents. Alternatively, fusion proteins comprising at least the antigen binding region of an antibody of the invention linked to at least a functionally active portion of an enzyme of the invention can be constructed using recombinant DNA techniques well known in the art (see, e.g., Neuberger et al., *Nature*, 312: 604-608 (1984)).

Further antibody modifications are contemplated. For example, the antibody may be linked to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol, polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. The antibody also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences*, 16th edition, Oslo, A., Ed., (1980). To increase the serum half

life of the antibody, one may incorporate a salvage receptor binding epitope into the antibody (especially an antibody fragment) as described in U.S. Patent 5,739,277, for example. As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (e.g., IgG₁, IgG₂, IgG₃, or IgG₄) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule.

B. ASSAY METHODS

Ligand/receptor binding studies may be carried out in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Cell-based assays and animal models can be used as diagnostic methods and to further understand the interaction between the ligands and receptors identified herein and the development and pathogenesis of the conditions and diseases referred to herein.

In one approach, mammalian cells may be transfected with the ligands or receptors described herein, and the ability of the agonists or antagonists to stimulate or inhibit binding or activity is analyzed. Suitable cells can be transfected with the desired gene, and monitored for activity. Such transfected cell lines can then be used to test the ability of antagonist(s) or agonist(s) to inhibit or stimulate, for example, to modulate B-cell proliferation or Ig secretion. Cells transfected with the coding sequence of the genes identified herein can further be used to identify drug candidates for the treatment of immune related diseases or cancer.

In addition, primary cultures derived from transgenic animals can be used in the cell-based assays. Techniques to derive continuous cell lines from transgenic animals are well known in the art. [see, e.g., Small et al., Mol. Cell. Biol., 5:642-648 (1985)].

One suitable cell based assay is the addition of epitope-tagged ligand (e.g., AP or Flag) to cells that have or express the respective receptor, and analysis of binding (in presence or absence or prospective antagonists) by FACS staining with anti-tag antibody. In another assay, the ability of an agonist or antagonist to inhibit the TALL-1 or APRIL induced proliferation of B cells is assayed. B cells or cell lines are cultured with TALL-1

or APRIL in the presence or absence of prospective agonists or antagonists and the proliferation of B cells can be measured by ^3H -thymidine incorporation or cell number.

The results of the cell based *in vitro* assays can be further
5 verified using *in vivo* animal models. A variety of well known animal models can be used to further understand the role of the agonists and antagonists identified herein in the development and pathogenesis of for instance, immune related disease or cancer, and to test the efficacy of the candidate therapeutic agents. The *in*
10 *vivo* nature of such models makes them particularly predictive of responses in human patients. Animal models of immune related diseases include both non-recombinant and recombinant (transgenic) animals. Non-recombinant animal models include, for example, rodent, e.g., murine models. Such models can be generated by
15 introducing cells into syngeneic mice using standard techniques, e.g. subcutaneous injection, tail vein injection, spleen implantation, intraperitoneal implantation, and implantation under the renal capsule.

Animal models, for example, for graft-versus-host disease are
20 known. Graft-versus-host disease occurs when immunocompetent cells are transplanted into immunosuppressed or tolerant patients. The donor cells recognize and respond to host antigens. The response can vary from life threatening severe inflammation to mild cases of diarrhea and weight loss. Graft-versus-host disease models provide
25 a means of assessing T cell reactivity against MHC antigens and minor transplant antigens. A suitable procedure is described in detail in Current Protocols in Immunology, unit 4.3.

An animal model for skin allograft rejection is a means of testing the ability of T cells to mediate *in vivo* tissue
30 destruction which is indicative of and a measure of their role in anti-viral and tumor immunity. The most common and accepted models use murine tail-skin grafts. Repeated experiments have shown that skin allograft rejection is mediated by T cells, helper T cells and killer-effector T cells, and not antibodies. [Auchincloss, H.
35 Jr. and Sachs, D. H., Fundamental Immunology, 2nd ed., W. E. Paul ed., Raven Press, NY, 1989, 889-992]. A suitable procedure is described in detail in Current Protocols in Immunology, unit 4.4.

Other transplant rejection models which can be used to test the compositions of the invention are the allogeneic heart transplant models described by Tanabe, M. et al., Transplantation, (1994) 58:23 and Tinubu, S. A. et al., J. Immunol., (1994) 4330-4338.

5 Animal models for delayed type hypersensitivity provides an assay of cell mediated immune function as well. Delayed type hypersensitivity reactions are a T cell mediated in vivo immune response characterized by inflammation which does not reach a peak until after a period of time has elapsed after challenge with an
10 antigen. These reactions also occur in tissue specific autoimmune diseases such as multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE, a model for MS). A suitable procedure is described in detail in Current Protocols in Immunology, unit 4.5.

15 An animal model for arthritis is collagen-induced arthritis. This model shares clinical, histological and immunological characteristics of human autoimmune rheumatoid arthritis and is an acceptable model for human autoimmune arthritis. Mouse and rat models are characterized by synovitis, erosion of cartilage and
20 subchondral bone. The compounds of the invention can be tested for activity against autoimmune arthritis using the protocols described in Current Protocols in Immunology, above, units 15.5. See also the model using a monoclonal antibody to CD18 and VLA-4 integrins described in Issekutz, A. C. et al., Immunology, (1996) 88:569.

25 A model of asthma has been described in which antigen-induced airway hyper-reactivity, pulmonary eosinophilia and inflammation are induced by sensitizing an animal with ovalbumin and then challenging the animal with the same protein delivered by aerosol. Several animal models (guinea pig, rat, non-human primate) show
30 symptoms similar to atopic asthma in humans upon challenge with aerosol antigens. Murine models have many of the features of human asthma. Suitable procedures to test the compositions of the invention for activity and effectiveness in the treatment of asthma are described by Wolyniec, W. W. et al., Am. J. Respir. Cell Mol. Biol., (1998) 18:777 and the references cited therein.
35

 Additionally, the compositions of the invention can be tested on animal models for psoriasis like diseases. The compounds of the invention can be tested in the scid/scid mouse model described by

Schon, M. P. et al., Nat. Med., (1997) 3:183, in which the mice demonstrate histopathologic skin lesions resembling psoriasis. Another suitable model is the human skin/scid mouse chimera prepared as described by Nickoloff, B. J. et al., Am. J. Path.,
5 (1995) 146:580.

Various animal models are well known for testing anti-cancer activity of a candidate therapeutic composition. These include human tumor xenografting into athymic nude mice or scid/scid mice, or genetic murine tumor models such as p53 knockout mice.

10 Recombinant (transgenic) animal models can be engineered by introducing the coding portion of the molecules identified herein into the genome of animals of interest, using standard techniques for producing transgenic animals. Animals that can serve as a target for transgenic manipulation include, without limitation,
15 mice, rats, rabbits, guinea pigs, sheep, goats, pigs, and non-human primates, e.g. baboons, chimpanzees and monkeys. Techniques known in the art to introduce a transgene into such animals include pronucleic microinjection (Hoppe and Wanger, U.S. Patent No. 4,873,191); retrovirus-mediated gene transfer into germ lines
20 (e.g., Van der Putten et al., Proc. Natl. Acad. Sci. USA, 82, 6148-615 [1985]); gene targeting in embryonic stem cells (Thompson et al., Cell, 56, 313-321 [1989]); electroporation of embryos (Lo, Mol. Cel. Biol., 3, 1803-1814 [1983]); sperm-mediated gene transfer (Lavitrano et al., Cell, 57, 717-73 [1989]). For review, see, for
25 example, U.S. Patent No. 4,736,866.

For the purpose of the present invention, transgenic animals include those that carry the transgene only in part of their cells ("mosaic animals"). The transgene can be integrated either as a single transgene, or in concatamers, e.g., head-to-head or head-to-
30 tail tandems. Selective introduction of a transgene into a particular cell type is also possible by following, for example, the technique of Lasko et al., Proc. Natl. Acad. Sci. USA, 89, 6232-636 (1992).

The expression of the transgene in transgenic animals can be
35 monitored by standard techniques. For example, Southern blot analysis or PCR amplification can be used to verify the integration of the transgene. The level of mRNA expression can then be analyzed using techniques such as *in situ* hybridization, Northern

blot analysis, PCR, or immunocytochemistry. The animals may be further examined for signs of immune disease pathology, for example by histological examination to determine infiltration of immune cells into specific tissues or for the presence of cancerous or malignant tissue.

Alternatively, "knock out" animals can be constructed which have a defective or altered gene encoding a polypeptide identified herein, as a result of homologous recombination between the endogenous gene encoding the polypeptide and altered genomic DNA encoding the same polypeptide introduced into an embryonic cell of the animal. For example, cDNA encoding a particular polypeptide can be used to clone genomic DNA encoding that polypeptide in accordance with established techniques. A portion of the genomic DNA encoding a particular polypeptide can be deleted or replaced with another gene, such as a gene encoding a selectable marker which can be used to monitor integration. Typically, several kilobases of unaltered flanking DNA (both at the 5' and 3' ends) are included in the vector [see e.g., Thomas and Capecchi, Cell, 51:503 (1987) for a description of homologous recombination vectors]. The vector is introduced into an embryonic stem cell line (e.g., by electroporation) and cells in which the introduced DNA has homologously recombined with the endogenous DNA are selected [see e.g., Li et al., Cell, 69:915 (1992)]. The selected cells are then injected into a blastocyst of an animal (e.g., a mouse or rat) to form aggregation chimeras [see e.g., Bradley, in *Teratocarcinomas and Embryonic Stem Cells: A Practical Approach*, E. J. Robertson, ed. (IRL, Oxford, 1987), pp. 113-152]. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term to create a "knock out" animal. Progeny harboring the homologously recombined DNA in their germ cells can be identified by standard techniques and used to breed animals in which all cells of the animal contain the homologously recombined DNA. Knockout animals can be characterized for instance, for their ability to defend against certain pathological conditions and for their development of pathological conditions due to absence of the polypeptide.

C. FORMULATIONS

The TACI antibodies described herein, are optionally employed in a carrier. Suitable carriers and their formulations are described in Remington's Pharmaceutical Sciences, 16th ed., 1980, Mack Publishing Co., edited by Osol et al. Typically, an appropriate amount of a pharmaceutically-acceptable salt is used in the carrier to render the formulation isotonic. Examples of the carrier include saline, Ringer's solution and dextrose solution. The pH of the carrier is preferably from about 5 to about 8, and more preferably from about 7.4 to about 7.8. It will be apparent to those persons skilled in the art that certain carriers may be more preferable depending upon, for instance, the route of administration and concentration of active agent being administered. The carrier may be in the form of a lyophilized formulation or aqueous solution.

Acceptable carriers, excipients, or stabilizers are preferably nontoxic to cells and/or recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; and/or non-ionic surfactants such as TWEENTM, PLURONICSTM or polyethylene glycol (PEG).

The formulation may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other.

The TACI antibodies described herein, may also be entrapped in microcapsules prepared, for example, by coacervation techniques or

by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

The formulations to be used for in vivo administration should be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the active agent, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

D. MODES OF THERAPY

The molecules described herein are useful in treating various pathological conditions, such as immune related diseases or cancer. These conditions can be treated by stimulating or inhibiting a selected activity associated with TALL-1, APRIL, TACI, BCMA, TACIs or BR3 in a mammal through, for example, administration of one or more TACI antibodies or antagonists or agonists described herein.

Diagnosis in mammals of the various pathological conditions described herein can be made by the skilled practitioner. Diagnostic techniques are available in the art which allow, e.g., for the diagnosis or detection of cancer or immune related disease in a mammal. For instance, cancers may be identified through

techniques, including but not limited to, palpation, blood analysis, x-ray, NMR and the like. Immune related diseases can also be readily identified. In systemic lupus erythematosus, the central mediator of disease is the production of auto-reactive antibodies to self proteins/tissues and the subsequent generation of immune-mediated inflammation. Multiple organs and systems are affected clinically including kidney, lung, musculoskeletal system, mucocutaneous, eye, central nervous system, cardiovascular system, gastrointestinal tract, bone marrow and blood.

10 Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disease that mainly involves the synovial membrane of multiple joints with resultant injury to the articular cartilage. The pathogenesis is T lymphocyte dependent and is associated with the production of rheumatoid factors, auto-antibodies directed against self IgG, with the resultant formation of immune complexes that attain high levels in joint fluid and blood. These complexes in the joint may induce the marked infiltrate of lymphocytes and monocytes into the synovium and subsequent marked synovial changes; the joint space/fluid is infiltrated by similar cells with the addition of numerous neutrophils. Tissues affected are primarily the joints, often in symmetrical pattern. However, extra-articular disease also occurs in two major forms. One form is the development of extra-articular lesions with ongoing progressive joint disease and typical lesions of pulmonary fibrosis, vasculitis, and cutaneous ulcers. The second form of extra-articular disease is the so called Felty's syndrome which occurs late in the RA disease course, sometimes after joint disease has become quiescent, and involves the presence of neutropenia, thrombocytopenia and splenomegaly. This can be accompanied by vasculitis in multiple organs with formations of infarcts, skin ulcers and gangrene. Patients often also develop rheumatoid nodules in the subcutis tissue overlying affected joints; the nodules late stage have necrotic centers surrounded by a mixed inflammatory cell infiltrate. Other manifestations which can occur in RA include: pericarditis, pleuritis, coronary arteritis, interstitial pneumonitis with pulmonary fibrosis, keratoconjunctivitis sicca, and rheumatoid nodules.

Juvenile chronic arthritis is a chronic idiopathic

inflammatory disease which begins often at less than 16 years of age. Its phenotype has some similarities to RA; some patients which are rheumatoid factor positive are classified as juvenile rheumatoid arthritis. The disease is sub-classified into three
5 major categories: pauciarticular, polyarticular, and systemic. The arthritis can be severe and is typically destructive and leads to joint ankylosis and retarded growth. Other manifestations can include chronic anterior uveitis and systemic amyloidosis.

Spondyloarthropathies are a group of disorders with some
10 common clinical features and the common association with the expression of HLA-B27 gene product. The disorders include: ankylosing spondylitis, Reiter's syndrome (reactive arthritis), arthritis associated with inflammatory bowel disease, spondylitis associated with psoriasis, juvenile onset spondyloarthropathy and
15 undifferentiated spondyloarthropathy. Distinguishing features include sacroileitis with or without spondylitis; inflammatory asymmetric arthritis; association with HLA-B27 (a serologically defined allele of the HLA-B locus of class I MHC); ocular inflammation, and absence of autoantibodies associated with other
20 rheumatoid disease. The cell most implicated as key to induction of the disease is the CD8+ T lymphocyte, a cell which targets antigen presented by class I MHC molecules. CD8+ T cells may react against the class I MHC allele HLA-B27 as if it were a foreign peptide expressed by MHC class I molecules. It has been
25 hypothesized that an epitope of HLA-B27 may mimic a bacterial or other microbial antigenic epitope and thus induce a CD8+ T cells response.

Systemic sclerosis (scleroderma) has an unknown etiology. A hallmark of the disease is induration of the skin; likely this is
30 induced by an active inflammatory process. Scleroderma can be localized or systemic; vascular lesions are common and endothelial cell injury in the microvasculature is an early and important event in the development of systemic sclerosis; the vascular injury may be immune mediated. An immunologic basis is implied by the
35 presence of mononuclear cell infiltrates in the cutaneous lesions and the presence of anti-nuclear antibodies in many patients. ICAM-1 is often upregulated on the cell surface of fibroblasts in skin lesions suggesting that T cell interaction with these cells

may have a role in the pathogenesis of the disease. Other organs involved include: the gastrointestinal tract: smooth muscle atrophy and fibrosis resulting in abnormal peristalsis/motility; kidney: concentric subendothelial intimal proliferation affecting small
5 arcuate and interlobular arteries with resultant reduced renal cortical blood flow, results in proteinuria, azotemia and hypertension; skeletal muscle: atrophy, interstitial fibrosis; inflammation; lung: interstitial pneumonitis and interstitial fibrosis; and heart: contraction band necrosis, scarring/fibrosis.

10 Idiopathic inflammatory myopathies including dermatomyositis, polymyositis and others are disorders of chronic muscle inflammation of unknown etiology resulting in muscle weakness. Muscle injury/inflammation is often symmetric and progressive. Autoantibodies are associated with most forms. These myositis-
15 specific autoantibodies are directed against and inhibit the function of components, proteins and RNA's, involved in protein synthesis.

Sjogren's syndrome is due to immune-mediated inflammation and subsequent functional destruction of the tear glands and salivary
20 glands. The disease can be associated with or accompanied by inflammatory connective tissue diseases. The disease is associated with autoantibody production against Ro and La antigens, both of which are small RNA-protein complexes. Lesions result in keratoconjunctivitis sicca, xerostomia, with other manifestations
25 or associations including biliary cirrhosis, peripheral or sensory neuropathy, and palpable purpura.

Systemic vasculitis are diseases in which the primary lesion is inflammation and subsequent damage to blood vessels which results in ischemia/necrosis/degeneration to tissues supplied by
30 the affected vessels and eventual end-organ dysfunction in some cases. Vasculitides can also occur as a secondary lesion or sequelae to other immune-inflammatory mediated diseases such as rheumatoid arthritis, systemic sclerosis, etc., particularly in diseases also associated with the formation of immune complexes.
35 Diseases in the primary systemic vasculitis group include: systemic necrotizing vasculitis: polyarteritis nodosa, allergic angiitis and granulomatosis, polyangiitis; Wegener's granulomatosis; lymphomatoid granulomatosis; and giant cell arteritis.

Miscellaneous vasculitides include: mucocutaneous lymph node syndrome (MLNS or Kawasaki's disease), isolated CNS vasculitis, Behet's disease, thromboangiitis obliterans (Buerger's disease) and cutaneous necrotizing venulitis. The pathogenic mechanism of most of the types of vasculitis listed is believed to be primarily due to the deposition of immunoglobulin complexes in the vessel wall and subsequent induction of an inflammatory response either via ADCC, complement activation, or both.

Sarcoidosis is a condition of unknown etiology which is characterized by the presence of epithelioid granulomas in nearly any tissue in the body; involvement of the lung is most common. The pathogenesis involves the persistence of activated macrophages and lymphoid cells at sites of the disease with subsequent chronic sequelae resultant from the release of locally and systemically active products released by these cell types.

Autoimmune hemolytic anemia including autoimmune hemolytic anemia, immune pancytopenia, and paroxysmal nocturnal hemoglobinuria is a result of production of antibodies that react with antigens expressed on the surface of red blood cells (and in some cases other blood cells including platelets as well) and is a reflection of the removal of those antibody coated cells via complement mediated lysis and/or ADCC/Fc-receptor-mediated mechanisms.

In autoimmune thrombocytopenia including thrombocytopenic purpura, and immune-mediated thrombocytopenia in other clinical settings, platelet destruction/removal occurs as a result of either antibody or complement attaching to platelets and subsequent removal by complement lysis, ADCC or FC-receptor mediated mechanisms.

Thyroiditis including Grave's disease, Hashimoto's thyroiditis, juvenile lymphocytic thyroiditis, and atrophic thyroiditis, are the result of an autoimmune response against thyroid antigens with production of antibodies that react with proteins present in and often specific for the thyroid gland. Experimental models exist including spontaneous models: rats (BUF and BB rats) and chickens (obese chicken strain); inducible models: immunization of animals with either thyroglobulin, thyroid microsomal antigen (thyroid peroxidase).

Type I diabetes mellitus or insulin-dependent diabetes is the

autoimmune destruction of pancreatic islet β cells; this destruction is mediated by auto-antibodies and auto-reactive T cells. Antibodies to insulin or the insulin receptor can also produce the phenotype of insulin-non-responsiveness.

5 Immune mediated renal diseases, including glomerulonephritis and tubulointerstitial nephritis, are the result of antibody or T lymphocyte mediated injury to renal tissue either directly as a result of the production of autoreactive antibodies or T cells against renal antigens or indirectly as a result of the deposition
10 of antibodies and/or immune complexes in the kidney that are reactive against other, non-renal antigens. Thus other immune-mediated diseases that result in the formation of immune-complexes can also induce immune mediated renal disease as an indirect sequelae. Both direct and indirect immune mechanisms result in
15 inflammatory response that produces/induces lesion development in renal tissues with resultant organ function impairment and in some cases progression to renal failure. Both humoral and cellular immune mechanisms can be involved in the pathogenesis of lesions.

 Demyelinating diseases of the central and peripheral nervous
20 systems, including Multiple Sclerosis; idiopathic demyelinating polyneuropathy or Guillain-Barr syndrome; and Chronic Inflammatory Demyelinating Polyneuropathy, are believed to have an autoimmune basis and result in nerve demyelination as a result of damage caused to oligodendrocytes or to myelin directly. In MS there is
25 evidence to suggest that disease induction and progression is dependent on T lymphocytes. Multiple Sclerosis is a demyelinating disease that is T lymphocyte-dependent and has either a relapsing-remitting course or a chronic progressive course. The etiology is unknown; however, viral infections, genetic predisposition,
30 environment, and autoimmunity all contribute. Lesions contain infiltrates of predominantly T lymphocyte mediated, microglial cells and infiltrating macrophages; CD4+T lymphocytes are the predominant cell type at lesions. The mechanism of oligodendrocyte cell death and subsequent demyelination is not known but is likely
35 T lymphocyte driven.

 Inflammatory and Fibrotic Lung Disease, including Eosinophilic Pneumonias; Idiopathic Pulmonary Fibrosis, and Hypersensitivity Pneumonitis may involve a dysregulated immune-inflammatory

response. Inhibition of that response would be of therapeutic benefit.

Autoimmune or Immune-mediated Skin Disease including Bullous Skin Diseases, Erythema Multiforme, and Contact Dermatitis are
5 mediated by auto-antibodies, the genesis of which is T lymphocyte-dependent.

Psoriasis is a T lymphocyte-mediated inflammatory disease. Lesions contain infiltrates of T lymphocytes, macrophages and antigen processing cells, and some neutrophils.

10 Allergic diseases, including asthma, allergic rhinitis; atopic dermatitis; food hypersensitivity; and urticaria are T lymphocyte dependent. These diseases are predominantly mediated by T lymphocyte induced inflammation, IgE mediated-inflammation or a combination of both.

15 Transplantation associated diseases, including Graft rejection and Graft-Versus-Host-Disease (GVHD) are T lymphocyte-dependent; inhibition of T lymphocyte function is ameliorative.

Other diseases in which intervention of the immune and/or inflammatory response have benefit are Infectious disease including
20 but not limited to viral infection (including but not limited to AIDS, hepatitis A, B, C, D, E) bacterial infection, fungal infections, and protozoal and parasitic infections (molecules (or derivatives/agonists) which stimulate the MLR can be utilized therapeutically to enhance the immune response to infectious
25 agents), diseases of immunodeficiency (molecules/derivatives/agonists) which stimulate the MLR can be utilized therapeutically to enhance the immune response for conditions of inherited, acquired, infectious induced (as in HIV infection), or iatrogenic (i.e. as from chemotherapy)
30 immunodeficiency), and neoplasia.

The TACI antibodies or antagonist(s) or agonist(s) can be administered in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal,
35 subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. Optionally, administration may be performed through mini-pump infusion using various commercially available devices. The antagonists or agonists may also be

employed using gene therapy techniques which have been described in the art.

Effective dosages and schedules for administering TACI antibodies or antagonists or agonists may be determined empirically, and making such determinations is within the skill in the art. Single or multiple dosages may be employed. It is presently believed that an effective dosage or amount of antagonist or agonist used alone may range from about 1 ng/kg to about 100 mg/kg of body weight or more per day. Interspecies scaling of dosages can be performed in a manner known in the art, e.g., as disclosed in Mordenti et al., Pharmaceut. Res., 8:1351 (1991).

When *in vivo* administration of a TACI antibody or an agonist or antagonist thereof is employed, normal dosage amounts may vary from about 10 ng/kg to up to 100 mg/kg of mammal body weight or more per day, preferably about 1 µg/kg/day to 10 mg/kg/day, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature; see, for example, U.S. Pat. Nos. 4,657,760; 5,206,344; or 5,225,212. It is anticipated that different formulations will be effective for different treatment compounds and different disorders, that administration targeting one organ or tissue, for example, may necessitate delivery in a manner different from that to another organ or tissue. Those skilled in the art will understand that the dosage that must be administered will vary depending on, for example, the mammal which will receive the therapy, the route of administration, and other drugs or therapies being administered to the mammal.

Depending on the type of cells and/or severity of the disease, about 1 µg/kg to 150 mg/kg (e.g. 0.1-20 mg/kg) of antagonist antibody or agonist antibody is an initial candidate dosage for administration, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful.

Optionally, prior to administration of any therapy, the mammal or patient can be tested to determine levels or activity of TALL-1, APRIL, TACI, BCMA, TACIs or BR3. Such testing may be conducted by ELISA or FACS of serum samples or peripheral blood leukocytes.

5 A single type of therapy may be used in the methods of the invention. For example, a TACI antibody may be administered. Alternatively, the skilled practitioner may opt to employ a combination of TACI antibodies and antagonists or agonists in the methods, e.g., a combination of a TACI antibody and a BR3 antibody.
10 It may further be desirable to employ a dual agonist or antagonist, i.e., such as an antagonist which acts to block or inhibit both TALL-1 and APRIL. Such an antagonist molecule may, for instance, bind to epitopes conserved between TALL-1 and APRIL, or TACI, TACIs, BR3, and BCMA.

15 It is contemplated that yet additional therapies may be employed in the methods. The one or more other therapies may include but are not limited to, administration of radiation therapy, cytokine(s), growth inhibitory agent(s), chemotherapeutic agent(s), cytotoxic agent(s), tyrosine kinase inhibitors, ras farnesyl
20 transferase inhibitors, angiogenesis inhibitors, and cyclin-dependent kinase inhibitors which are known in the art and defined further with particularity in Section I above. In addition, therapies based on therapeutic antibodies that target tumor antigens such as Rituxan™ or Herceptin™ as well as anti-angiogenic
25 antibodies such as anti-VEGF.

Preparation and dosing schedules for chemotherapeutic agents may be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in
30 *Chemotherapy Service* Ed., M.C. Perry, Williams & Wilkins, Baltimore, MD (1992). The chemotherapeutic agent may precede, or follow administration of, e.g. an agonist or antagonist, or may be given simultaneously therewith. The agonist or antagonist, for instance, may also be combined with an anti-oestrogen compound such
35 as tamoxifen or an anti-progesterone such as onapristone (see, EP 616812) in dosages known for such molecules.

It may be desirable to also administer antibodies against other antigens, such as antibodies which bind to CD20, CD11a, CD18,

CD40, ErbB2, EGFR, ErbB3, ErbB4, vascular endothelial factor (VEGF), or other TNFR family members (such as DR4, DR5, OPG, TNFR1, TNFR2). Alternatively, or in addition, two or more antibodies binding the same or two or more different antigens disclosed herein may be co-administered to the patient. Sometimes, it may be beneficial to also administer one or more cytokines to the patient. In one embodiment, the agonists or antagonists herein are co-administered with a growth inhibitory agent. For example, the growth inhibitory agent may be administered first, followed by an agonist or antagonist of the present invention.

The antagonist or agonist (and one or more other therapies) may be administered concurrently or sequentially. Following administration of antagonist or agonist, treated cells *in vitro* can be analyzed. Where there has been *in vivo* treatment, a treated mammal can be monitored in various ways well known to the skilled practitioner. For instance, markers of B cell activity such as Ig production (non-specific or antigen specific) can be assayed.

E. METHODS OF RECOMBINANT PRODUCTION

The invention also provides isolated nucleic acids encoding TACI antibodies as disclosed herein, vectors and host cells comprising the nucleic acid, and recombinant techniques for the production of the antibody.

For recombinant production of the antibody, the nucleic acid encoding it is isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the antibody is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the antibody). Many vectors are available. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence.

The methods herein include methods for the production of chimeric or recombinant anti-TACI antibodies which comprise the steps of providing a vector comprising a DNA sequence encoding an anti-TACI antibody light chain or heavy chain (or both a light

chain and a heavy chain), transfecting or transforming a host cell with the vector, and culturing the host cell(s) under conditions sufficient to produce the recombinant anti-TACI antibody product.

(i) *Signal sequence component*

5 The anti-TACI antibody of this invention may be produced recombinantly not only directly, but also as a fusion polypeptide with a heterologous polypeptide, which is preferably a signal sequence or other polypeptide having a specific cleavage site at the N-terminus of the mature protein or polypeptide. The
10 heterologous signal sequence selected preferably is one that is recognized and processed (i.e., cleaved by a signal peptidase) by the host cell. For prokaryotic host cells that do not recognize and process the native antibody signal sequence, the signal sequence is substituted by a prokaryotic signal sequence selected,
15 for example, from the group of the alkaline phosphatase, penicillinase, lpp, or heat-stable enterotoxin II leaders. For yeast secretion the native signal sequence may be substituted by, e.g., the yeast invertase leader, α factor leader (including
20 *Saccharomyces* and *Kluyveromyces* α -factor leaders), or acid phosphatase leader, the *C. albicans* glucoamylase leader, or the signal described in WO 90/13646. In mammalian cell expression, mammalian signal sequences as well as viral secretory leaders, for example, the herpes simplex gD signal, are available.

25 The DNA for such precursor region is ligated in reading frame to DNA encoding the antibody.

(ii) *Origin of replication component*

Both expression and cloning vectors contain a nucleic acid sequence that enables the vector to replicate in one or more selected host cells. Generally, in cloning vectors this sequence
30 is one that enables the vector to replicate independently of the host chromosomal DNA, and includes origins of replication or autonomously replicating sequences. Such sequences are well known for a variety of bacteria, yeast, and viruses. The origin of replication from the plasmid pBR322 is suitable for most Gram-
35 negative bacteria, the 2 μ plasmid origin is suitable for yeast, and various viral origins (SV40, polyoma, adenovirus, VSV or BPV) are useful for cloning vectors in mammalian cells. Generally, the

origin of replication component is not needed for mammalian expression vectors (the SV40 origin may typically be used only because it contains the early promoter).

(iii) Selection gene component

5 Expression and cloning vectors may contain a selection gene, also termed a selectable marker. Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins, e.g., ampicillin, neomycin, methotrexate, or tetracycline, (b) complement auxotrophic deficiencies, or (c) supply critical
10 nutrients not available from complex media, e.g., the gene encoding D-alanine racemase for *Bacilli*.

One example of a selection scheme utilizes a drug to arrest growth of a host cell. Those cells that are successfully transformed with a heterologous gene produce a protein conferring
15 drug resistance and thus survive the selection regimen. Examples of such dominant selection use the drugs neomycin, mycophenolic acid and hygromycin.

Another example of suitable selectable markers for mammalian cells are those that enable the identification of cells competent
20 to take up the antibody nucleic acid, such as DHFR, thymidine kinase, metallothionein-I and -II, preferably primate metallothionein genes, adenosine deaminase, ornithine decarboxylase, etc.

For example, cells transformed with the DHFR selection gene
25 are first identified by culturing all of the transformants in a culture medium that contains methotrexate (Mtx), a competitive antagonist of DHFR. An appropriate host cell when wild-type DHFR is employed is the Chinese hamster ovary (CHO) cell line deficient in DHFR activity.

30 Alternatively, host cells (particularly wild-type hosts that contain endogenous DHFR) transformed or co-transformed with DNA sequences encoding the anti-DR4 antibody, wild-type DHFR protein, and another selectable marker such as aminoglycoside 3'-phosphotransferase (APH) can be selected by cell growth in medium
35 containing a selection agent for the selectable marker such as an aminoglycosidic antibiotic, e.g., kanamycin, neomycin, or G418. See U.S. Patent No. 4,965,199.

A suitable selection gene for use in yeast is the *trp1* gene

present in the yeast plasmid YRp7 (Stinchcomb et al., *Nature*, 282:39 (1979)). The *trp1* gene provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example, ATCC No. 44076 or PEP4-1. Jones, *Genetics*, 85:12
5 (1977): The presence of the *trp1* lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan. Similarly, *Leu2*-deficient yeast strains (ATCC 20,622 or 38,626) are complemented by known plasmids bearing the *Leu2* gene.

10 In addition, vectors derived from the 1.6 μ m circular plasmid pKD1 can be used for transformation of *Kluyveromyces* yeasts. Alternatively, an expression system for large-scale production of recombinant calf chymosin was reported for *K. lactis*. Van den Berg, *Bio/Technology*, 8:135 (1990). Stable multi-copy expression
15 vectors for secretion of mature recombinant human serum albumin by industrial strains of *Kluyveromyces* have also been disclosed. Fleer et al., *Bio/Technology*, 9:968-975 (1991).

(iv) Promoter component

Expression and cloning vectors usually contain a promoter that
20 is recognized by the host organism and is operably linked to the antibody nucleic acid. Promoters suitable for use with prokaryotic hosts include the *phoA* promoter, β -lactamase and lactose promoter systems, alkaline phosphatase, a tryptophan (*trp*) promoter system, and hybrid promoters such as the *tac* promoter. However, other
25 known bacterial promoters are suitable. Promoters for use in bacterial systems also will contain a Shine-Dalgarno (S.D.) sequence operably linked to the DNA encoding the anti-TACI antibody.

Promoter sequences are known for eukaryotes. Virtually all
30 eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription is initiated. Another sequence found 70 to 80 bases upstream from the start of transcription of many genes is a CNCAAT region where N may be any nucleotide. At the 3' end of most eukaryotic genes is an AATAAA
35 sequence that may be the signal for addition of the poly A tail to the 3' end of the coding sequence. All of these sequences are suitably inserted into eukaryotic expression vectors.

Examples of suitable promoting sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase or other glycolytic enzymes, such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phospho-
5 fructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase.

Other yeast promoters, which are inducible promoters having the additional advantage of transcription controlled by growth
10 conditions, are the promoter regions for alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, degradative enzymes associated with nitrogen metabolism, metallothionein, glyceraldehyde-3-phosphate dehydrogenase, and enzymes responsible for maltose and galactose utilization. Suitable vectors and promoters for use in
15 yeast expression are further described in EP 73,657. Yeast enhancers also are advantageously used with yeast promoters.

Anti-TACI antibody transcription from vectors in mammalian host cells is controlled, for example, by promoters obtained from the genomes of viruses such as polyoma virus, fowlpox virus,
20 adenovirus (such as Adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus, a retrovirus, hepatitis-B virus and most preferably Simian Virus 40 (SV40), from heterologous mammalian promoters, e.g., the actin promoter or an immunoglobulin promoter, from heat-shock promoters, provided such promoters are compatible
25 with the host cell systems.

The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment that also contains the SV40 viral origin of replication. The immediate early promoter of the human cytomegalovirus is conveniently obtained as a
30 HindIII E restriction fragment. A system for expressing DNA in mammalian hosts using the bovine papilloma virus as a vector is disclosed in U.S. Patent No. 4,419,446. A modification of this system is described in U.S. Patent No. 4,601,978. See also Reyes et al., Nature 297:598-601 (1982) on expression of human β -
35 interferon cDNA in mouse cells under the control of a thymidine kinase promoter from herpes simplex virus. Alternatively, the rous sarcoma virus long terminal repeat can be used as the promoter.

(v) *Enhancer element component*

Transcription of a DNA encoding the anti-TACI antibody of this invention by higher eukaryotes is often increased by inserting an enhancer sequence into the vector. Many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, α -fetoprotein, and insulin). Typically, however, one will use an enhancer from a eukaryotic cell virus. Examples include the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers. See also Yaniv, *Nature* 297:17-18 (1982) on enhancing elements for activation of eukaryotic promoters. The enhancer may be spliced into the vector at a position 5' or 3' to the antibody-encoding sequence, but is preferably located at a site 5' from the promoter.

15 (vi) *Transcription termination component*

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human, or nucleated cells from other multicellular organisms) will also contain sequences necessary for the termination of transcription and for stabilizing the mRNA. Such sequences are commonly available from the 5' and, occasionally 3', untranslated regions of eukaryotic or viral DNAs or cDNAs. These regions contain nucleotide segments transcribed as polyadenylated fragments in the untranslated portion of the mRNA encoding the multivalent antibody. One useful transcription termination component is the bovine growth hormone polyadenylation region. See WO94/11026 and the expression vector disclosed therein.

 (vii) *Selection and transformation of host cells*

Suitable host cells for cloning or expressing the DNA in the vectors herein are the prokaryote, yeast, or higher eukaryote cells described above. Suitable prokaryotes for this purpose include eubacteria, such as Gram-negative or Gram-positive organisms, for example, Enterobacteriaceae such as *Escherichia*, e.g., *E. coli*, *Enterobacter*, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, e.g., *Salmonella typhimurium*, *Serratia*, e.g., *Serratia marcescans*, and *Shigella*, as well as Bacilli such as *B. subtilis* and *B. licheniformis* (e.g., *B. licheniformis* 41P disclosed in DD 266,710

published 12 April 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*. One preferred *E. coli* cloning host is *E. coli* 294 (ATCC 31,446), although other strains such as *E. coli* B, *E. coli* X1776 (ATCC 31,537), and *E. coli* W3110 (ATCC 27,325) are suitable.

5 These examples are illustrative rather than limiting.

In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for TAC1 antibody-encoding vectors. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower
10 eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe*; *Kluyveromyces* hosts such as, e.g., *K. lactis*, *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC 16,045), *K. wickerhamii* (ATCC 24,178), *K. waltii* (ATCC 56,500), *K. drosophilae*
15 (ATCC 36,906), *K. thermotolerans*, and *K. marxianus*; *Yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070); *Candida*; *Trichoderma reesei* (EP 244,234); *Neurospora crassa*; *Schwanniomyces* such as *Schwanniomyces occidentalis*; and filamentous fungi such as, e.g., *Neurospora*, *Penicillium*, *Tolypocladium*, and *Aspergillus* hosts such
20 as *A. nidulans* and *A. niger*.

Suitable host cells for the expression of glycosylated antibody are derived from multicellular organisms. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive
25 insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruitfly), and *Bombyx mori* have been identified. A variety of viral strains for transfection are publicly available, e.g., the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such
30 viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

Plant cell cultures of cotton, corn, potato, soybean, petunia,
35 tomato, and tobacco can also be utilized as hosts.

However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has

become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., *J. Gen Virol.* 5 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); mouse sertoli cells (TM4, Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); 10 human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982)); 15 MRC 5 cells; FS4 cells; a human hepatoma line (Hep G2); and myeloma or lymphoma cells (e.g. Y0, J558L, P3 and NS0 cells) (see US Patent No. 5,807,715).

Host cells are transformed with the above-described expression or cloning vectors for antibody production and cultured in 20 conventional nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

(viii) *Culturing the host cells*

The host cells used to produce the antibody of this invention 25 may be cultured in a variety of media. Commercially available media such as Ham's F10 (Sigma), Minimal Essential Medium ((MEM), (Sigma), RPMI-1640 (Sigma), and Dulbecco's Modified Eagle's Medium ((DMEM), Sigma) are suitable for culturing the host cells. In addition, any of the media described in Ham et al., *Meth. Enz.* 30 58:44 (1979), Barnes et al., *Anal. Biochem.* 102:255 (1980), U.S. Pat. Nos. 4,767,704; 4,657,866; 4,927,762; 4,560,655; or 5,122,469; WO 90/03430; WO 87/00195; or U.S. Patent Re. 30,985 may be used as culture media for the host cells. Any of these media may be supplemented as necessary with hormones and/or other growth factors 35 (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleotides (such as adenosine and

thymidine), antibiotics (such as GENTAMYCIN™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also
5 be included at appropriate concentrations that would be known to those skilled in the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

10 (ix) Purification

When using recombinant techniques, the antibody can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the antibody is produced intracellularly, as a first step, the particulate debris, either
15 host cells or lysed fragments, is removed, for example, by centrifugation or ultrafiltration. Carter et al., *Bio/Technology* 10:163-167 (1992) describe a procedure for isolating antibodies which are secreted to the periplasmic space of *E. coli*. Briefly, cell paste is thawed in the presence of sodium acetate (pH 3.5),
20 EDTA, and phenylmethylsulfonylfluoride (PMSF) over about 30 min. Cell debris can be removed by centrifugation. Where the antibody is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore
25 Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

The antibody composition prepared from the cells can be
30 purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being the preferred purification technique. The suitability of protein A as an affinity ligand depends on the species and isotype of any immunoglobulin Fc region that is present
35 in the antibody. Protein A can be used to purify antibodies that are based on human $\gamma 1$, $\gamma 2$, or $\gamma 4$ heavy chains (Lindmark et al., *J. Immunol. Meth.* 62:1-13 (1983)). Protein G is recommended for all

mouse isotypes and for human $\gamma 3$ (Guss et al., *EMBO J.* 5:15671575 (1986)). The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or
5 poly(styrenediviny)benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the antibody comprises a C_H3 domain, the Bakerbond ABXTM resin (J. T. Baker, Phillipsburg, NJ) is useful for purification. Other techniques for protein purification such as fractionation on an
10 ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSETM chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromatofocusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody
15 to be recovered.

F. ARTICLES OF MANUFACTURE

In another embodiment of the invention, an article of manufacture containing materials useful for the treatment of the
20 disorders described above is provided. The article of manufacture comprises a container and a label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective
25 for treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The active agents in the composition may comprise antagonist(s) or agonist(s). The label on, or associated with, the container
30 indicates that the composition is used for treating the condition of choice. The article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from
35 a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

United States provisional application no. 60/398,530, filed July 25, 2002, and Seshasayee, D et al., (2003) Immunity 18:279-288 are hereby incorporated by reference in their entirety herein.

The following examples are offered by way of illustration and not by way of limitation. The disclosures of all citations in the specification are expressly incorporated herein by reference.

EXAMPLES

EXAMPLE 1:

10 Preparation of Anti-TACI Monoclonal Antibodies

Balb/c mice (obtained from Charles River Laboratories) were immunized by injecting 2µg of human TACI-IgG in MPL-TDM adjuvant (purchased from Ribi Immunochemical Research Inc., Hamilton, MT) 10 times into each hind foot pad. The human TACI-IgG immunoadhesin was prepared by methods described in Ashkenazi et al., Proc. Natl. Acad. Sci., 88:10535-10539 (1991). The immunoadhesin constructs consisted of amino acids 2-166 of the human TACI polypeptide. The TACI-ECD constructs were expressed in CHO cells using a heterologous signal sequence (pre-pro trypsin amino acids 1-17 of pCMV-1 Flag (Sigma)) and encoding the human IgG1 Fc region downstream of the TACI sequence, and then purified by protein A affinity chromatography.

Three days after the final boost, popliteal lymph nodes were removed from the mice and a single cell suspension was prepared in DMEM media (obtained from Biowhittaker Corp.) supplemented with 1% penicillin-streptomycin. The lymph node cells were then fused with murine myeloma cells P3X63AgU.1 (ATCC CRL 1597) using 35% polyethylene glycol and cultured in 96-well culture plates. Hybridomas resulting from the fusion were selected in HAT medium. Ten days after the fusion, hybridoma culture supernatants were screened in an ELISA to test for the presence of monoclonal antibodies binding to the TACI-IgG but not to CD4-IgG. The monoclonal antibodies were also screened for any binding to BMCA-IgG using the capture ELISA method.

For the capture ELISA, 96-well microtiter plates (Maxisorb; Nunc, Kamstrup, Denmark) were coated by adding 50 µl of 2.0 µg/ml goat anti-human IgG-Fc (Cappel Inc) in 50mM carbonate buffer, pH 9.6, to each well and incubating at 4°C overnight. Nonspecific

binding sites were blocked with 200 μ l of 2% BSA for 1 hour at room temperature. The plates were then washed three times with wash buffer (PBS containing 0.05% Tween 20). Following the wash steps, plates were incubated with 50 μ l/well of 0.4 μ g/ml TACI-IgG in PBS for 1 hour at room temperature. After washing 3 times 100 μ l of the hybridoma supernatants or various concentrations of polyclonal sera was added to designated wells. 100 μ l of P3X63AgU.1 myeloma cell conditioned medium was added to other designated wells as controls. The plates were incubated at room temperature for 1 hour on a shaker apparatus and then washed three times with wash buffer.

Next, 50 μ l HRP-conjugated goat anti-mouse IgG Fc (purchased from Cappel Laboratories), diluted 1:1000 in assay buffer (0.5% bovine serum albumin, 0.05% Tween-20, 0.01% Thimersol in PBS), was added to each well and the plates incubated for 1 hour at room temperature on a shaker apparatus. The plates were washed three times with wash buffer, followed by addition of 50 μ l of substrate (TMB microwell peroxidase substrate, Kirkegaard & Perry, Gaithersburg, MD) to each well and incubation at room temperature for 10 minutes. The reaction was stopped by adding 50 μ l of TMB 1-component stop solution (diethyl glycol, Kirkegaard & Perry) to each well, and absorbance at 450 nm was read in an automated microtiter plate reader.

The supernatants testing positive in the ELISA were then cloned twice by limiting dilution.

EXAMPLE 2

Identification of anti-TACI Antibodies that recognize membrane TACI

Anti-TACI antibodies designated 1G10.1.5, 5B6.3.10. and 6D11.3.1 were generated and prepared as discussed in Example 1 above. These mAbs recognized membrane TACI as determined by Flow cytometric analysis. Briefly, human B lymphoid IM9 cells (ATCC, CCL-159) (5×10^5 cells in 100 μ l of complete RPMI-1640 medium) were plated in 48-well microplates and were incubated overnight at 37°C in 5% CO₂ with 100 μ l of FITC-goat anti-mouse IgG Fc in 200 ml of binding buffer. After washing, the cells were then analyzed by FACScan.

The results of experiments showing that anti-TACI mAbs

recognized IM9 cells expression of TACI are shown in Figure 10.

EXAMPLE 3

Isotyping of anti-TACI Antibodies

5 The isotypes of the anti-TACI monoclonal antibodies (see Example 2 above) were determined by coating plates with isotype specific goat anti-mouse Ig (Fisher Biotech, Pittsburgh, PA) at 4° C overnight. After non-specific binding sites were blocked with 2% BSA, 100 µl of hybridoma culture supernatants or .5 µg/ml of
10 purified mAbs were added. After incubation for 30 minutes at room temperature, plates were incubated with HRP-conjugated goat anti-mouse Ig for 30 minutes at room temperature. The level of HRP bound to the plate was detected using HRP substrate as described above.

15 The anti-TACI antibodies, 1G10.1.5, 5B6.3.10. and 6D11.3.1, were found to be of the IgG1 isotype

EXAMPLE 4

Cross Reactivity Of Anti-TACI Mabs To Human BCMA

20 The potential cross reactivity of 1G10.1.5, 5B6.3.10 and 6D11.3.1 antibodies to human BCMA was also determined using the capture ELISA as described above with the following modification. Human BCMA-IgG molecules were captured to goat anti-human IgG-Fc coated microtiter wells. The BCMA-ECD immunoadhesins were prepared
25 by methods described in Ashkenazi et al., as cited above. The immunoadhesin constructs consisted of amino acids 5-51 of the human BCMA polypeptide. The BCMA-ECD constructs were expressed in CHO cells using a heterologous signal sequence (pre-pro trypsin amino acids 1-17 of pCMV-1 Flag (Sigma)) and encoding the human IgG1 Fc
30 region downstream of the BCMA sequence, and then purified by protein A affinity chromatography.

As shown in Figure 8, these anti-TACI mAbs failed to recognize BCMA-IgG in a capture ELISA.

EXAMPLE 5

Anti-TACI mAbs Block B cell Proliferation

An *in vitro* cell proliferation assay was conducted to determine

the effects of 1G10.1.5, 5B6.3.10 and 6D11.3.1 antibodies on B cells.

B cells were isolated from human peripheral blood using Lymphocyte Separation Medium (ICN) followed by purification using CD19+ MACS beads (Miltenyi Biotech). Enriched B cells were resuspended in complete medium (RPMI-1640, 10% fetal bovine serum, 2mM glutamine) and plated at 5×10^5 cells/well in tissue culture plates. The cells were then cultured at 37°C for 72 hours with 10 µg/ml anti-human CD40 antibody (BD Pharmingen), 100 ng/ml IL-4 (R&D Systems), and varying concentration of anti-TACI antibody. Anti-mouse IgG1 antibody (BD Pharmingen) was used as a control. Proliferation of B cells were measured by pulsing the cultures with methyl ³H-thymidine (1 µCi/well) for the last 6 hours of culture and then harvested. Thymidine incorporation was measured by scintillation counting.

The results are shown in Figure 9, and the proliferation of cells is reported as CPM $\times 10^{-3}$. The data shows that the anti-CD40 antibody induced B cell proliferation was inhibited in a dose dependent manner by the 6D11.3.1 and 5B6.3.10 anti-TACI antibodies. Other data from TACI knockout mice suggests that the TACI receptor is inhibitory in function, and in the absence of TACI, B cells may not receive inhibitory signals from TALL-1 (data not shown).

EXAMPLE 6

BlyS Binding to huTaci

For the Blys binding to huTACI ELISA, 96-well microtiter plates (Maxisorb; Nunc, Kamstrup, Denmark) were coated by adding 50 µl of 2.0 µg/ml goat anti-human IgG-Fc (Cappel Inc) in 50mM carbonate buffer, pH 9.6, to each well and incubating at 4°C overnight. Nonspecific binding sites were blocked with 200 µl of 2% BSA for 1 hr at RT. The plates were then washed three times with wash buffer (PBS containing 0.05% Tween 20). Following the wash steps, plates were incubated with 50 µl/well of 0.4 µg/ml TACI-IgG in assay buffer (0.5% bovine serum albumin, 0.05% Tween-20 in PBS). After washing 3 times 100 µl of the hybridoma supernatants or various concentrations of polyclonal sera was added to designated wells. 100 µl of P3X63AgU.1 myeloma cell conditioned medium was added to other designated wells as controls. The plates were incubated at

room temperature for 1 hour on a shaker apparatus and then washed three times with wash buffer.

Next, 100 µl of biotinylated human BlyS at 1:1600 in assay buffer was added to each well and the plates incubated for 1 hour at room temperature on a shaker apparatus and then washed three times with wash buffer. 50 µl Streptavidin-HRP (purchased from Zymed laboratory, CA), diluted 1:1000 in assay buffer (0.5% bovine serum albumin, 0.05% Tween-20 in PBS), was added to each well and the plates incubated for 1 hour at room temperature on a shaker apparatus. The plates were washed three times with wash buffer, followed by addition of 50 µl of substrate (TMB microwell peroxidase substrate, Kirkegard & Perry, Gaithersburg, MD) to each well and incubation at room temperature for 10 minutes. The reaction was stopped by adding 50 µl of TMB 1-component stop solution (diethyl glycol, Kirdegaard & Perry) to each well, and absorbance at 450 nm was read in an automated microtiter plate reader.

EXAMPLE 7

Inhibition of B cell proliferation by anti-TACI antibody that does not block BlyS binding to TACI in an ELISA assay

Other antibodies to TACI was generated in mouse and effects of the antibodies on signaling in human primary B cells were studied. Figure 11A demonstrates binding of three anti-TACI monoclonal antibodies, 6D11, 7B6.15.11, and 4C7.2.1, to 293 cells transfected with full-length human TACI. No binding of the TACI antibodies to mock-transfected 293 cells was observed (data not shown).

The antibodies were assayed for NF-kB activation activity. Human 293 cells were transfected with 0.1 µg of a full-length human TACI expression plasmid along with 1 µg of ELAM-luciferase reporter plasmid and 0.1 µg control pRL-TK plasmid (Promega Corporation). After 4 hr, indicated amounts of soluble recombinant human BlyS or TACI antibodies were added for 20 hr and reporter gene activity determined. Two out of three antibodies (6D11 and 7B6) displayed agonistic activity as evidenced by activation of the NFkB-luciferase reporter (dual-luciferase reporter assay system, Promega Corporation).

Variations in transfection efficiencies were controlled for by using equal amounts of protein and an internal Renilla reporter

control. In Figure 11B, the agonistic activity of two of the three antibodies (6D11 and 7B6) is shown. 6D11 and 7B6 were able to activate the NF- κ B reporter when compared to soluble human BLyS, which was used as a control. The third antibody 4C7 did not
5 stimulate reporter activity and is not an agonistic antibody. The 6D11 antibody blocked binding of BLyS to TACI; however, 7B6 and 4C7 did not (ELISA, data not shown).

The antibodies were tested in a human B-cell proliferation assay. 5×10^5 human B cells isolated from peripheral blood by
10 positive selection using magnetic beads (Lymphocyte Separation Medium, ICN Pharmaceuticals, followed by CD19+ MACS beads, Miltenyi Biotech) were stimulated with α -CD40 antibody (10 μ g/ml, BD Pharmingen) and IL-4 (100 ng/ml, R&D Systems) and increasing concentrations of two different clones of TACI agonistic antibodies
15 for 72 hr. [H3] counts are plotted as a function of TACI agonistic antibody concentration. All three antibodies are the same mouse isotype (IgG1) and 4C7 served as a matched isotype control antibody. The level of background B cell proliferation in the absence of any stimulus has been subtracted from each of the
20 indicated values in the graph. The two TACI agonistic antibodies 6D11 and 7B6 significantly inhibit B cell proliferation induced by α -CD40 antibody/IL4, while the nonagonistic antibody 4C7 does not. As shown in Figure 11C, α -CD40 antibody-induced B cell
25 proliferation was inhibited in a dose-dependent manner by the two agonistic monoclonal antibodies to TACI. All three antibodies are the same mouse isotype (IgG1), and 4C7 served as a matched isotype control antibody. The level of background B cell proliferation in the absence of any stimulus was subtracted from each of the
30 indicated values in the graph. The observation that both 6D11 and 7B6 could stimulate NF- κ B activity in 293 cells and inhibit B cell proliferation, while the nonagonistic antibody 4C7 could do neither, indicates that the observed effects on proliferation are due to an active inhibitory signal induced by TACI.

Deposit of Material

The following materials have been deposited with the American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209, USA (ATCC):

	<u>Material</u>	<u>ATCC Dep. No.</u>	<u>Deposit Date</u>
5	1G10.1.5	PTA-4297	May 7, 2002
	5B6.3.10	PTA-4298	May 7, 2002
	6D11.3.1	PTA-4299	May 7, 2002
	4C7.2.1	PTA-4999	February 11, 2003
10	7B6.15.11	PTA-5000	February 11, 2003

This deposit was made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposit will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Genentech, Inc. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC '122 and the Commissioner's rules pursuant thereto (including 37 CFR '1.14 with particular reference to 886 OG 638).

The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

The foregoing written description is considered to be sufficient to enable one skilled in the art to practice the

invention. The present invention is not to be limited in scope by the example presented herein. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing
5 description and fall within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. An antibody which specifically binds to a TACI receptor comprising amino acids 2 to 166 of SEQ ID NO: 3.

5

2. The antibody of claim 1, wherein the antibody does not bind BCMA receptor.

3. The antibody of claim 1, wherein the antibody is a
10 monoclonal antibody.

4. The monoclonal antibody of claim 3, wherein said monoclonal antibody comprises the 1G10.1.5 antibody secreted by the hybridoma deposited with ATCC as accession number PTA-4297; the 5B6.3.10
15 antibody secreted by the hybridoma deposited with ATCC as accession number PTA-4298, or the 6D11.3.1 antibody secreted by the hybridoma deposited with ATCC as accession number PTA-4299.

5. A monoclonal antibody which binds to the same epitope as
20 the epitope to which the 1G10.1.5 monoclonal antibody produced by the hybridoma cell line deposited as ATCC accession number PTA-4297 binds; the 5B6.3.10 monoclonal antibody produced by the hybridoma cell line deposited as ATCC accession number PTA-4298 binds; or the 6D11.3.1 monoclonal antibody produced by the hybridoma cell line
25 deposited as ATCC accession number PTA-4299 binds.

6. The hybridoma cell line which produces monoclonal antibody 1G10.1.5 and deposited with ATCC as accession number PTA-4297.

30 7. The monoclonal antibody 1G10.1.5 secreted by the hybridoma deposited with ATCC as accession number PTA-4297.

8. The hybridoma cell line which produces monoclonal antibody 5B6.3.10 and deposited with ATCC as accession number PTA-4298.

35

9. The monoclonal antibody 5B6.3.10 secreted by the hybridoma deposited with ATCC as accession number PTA-4298.

10. The hybridoma cell line which produces monoclonal antibody 6D11.3.1 and deposited with ATCC as accession number PTA-4299.

11. The monoclonal antibody 6D11.3.1 secreted by the hybridoma
5 deposited with ATCC as accession number PTA-4299.

12. An isolated anti-TACI receptor monoclonal antibody,
comprising an antibody which binds to TACI receptor comprising
amino acids 2 to 166 of SEQ ID NO: 3 and competitively inhibits
10 binding of the monoclonal antibody produced by the hybridoma
deposited as ATCC PTA-4297 to said TACI receptor.

13. An isolated anti-TACI receptor monoclonal antibody,
comprising an antibody which binds to TACI receptor comprising
15 amino acids 2 to 166 of SEQ ID NO: 3 and competitively inhibits
binding of the monoclonal antibody produced by the hybridoma
deposited as ATCC PTA-4298 to said TACI receptor.

14. An isolated anti-TACI receptor monoclonal antibody,
20 comprising an antibody which binds to TACI receptor comprising
amino acids 2 to 166 of SEQ ID NO: 3 and competitively inhibits
binding of the monoclonal antibody produced by the hybridoma
deposited as ATCC PTA-4299 to said TACI receptor.

25 15. A chimeric anti-TACI antibody which specifically binds to
TACI polypeptide and comprises (a) a sequence derived from the
1G10.1.5 antibody secreted by the hybridoma deposited with ATCC as
accession number PTA-4297; (b) a sequence derived from the 5B6.3.10
antibody secreted by the hybridoma deposited with ATCC as accession
30 number PTA-4298; or (c) a sequence derived from the 6D11.3.1
antibody secreted by the hybridoma deposited with ATCC as accession
number PTA-4299..

16. The anti-TACI antibody of claim 15 which is a humanized
35 antibody.

17. The anti-TACI receptor antibody of claim 1 which is linked
to one or more non-proteinaceous polymers selected from the group

consisting of polyethylene glycol, polypropylene glycol, and polyoxyalkylene.

18. The anti-TACI receptor antibody of claim 1 which is linked
5 to a cytotoxic agent or enzyme.

19. The anti-TACI receptor antibody of claim 1 which is linked
to a radioisotope, fluorescent compound or chemiluminescent
compound.

10

20. The anti-TACI receptor antibody of claim 1 which is
glycosylated.

21. The anti-TACI receptor antibody of claim 1 which is
15 unglycosylated.

22. A method of modulating TALL-1 or TACI polypeptide
biological activity in mammalian cells, comprising exposing said
mammalian cells to an effective amount of TACI receptor antibody;
20 wherein said antibody specifically binds to TACI receptor
comprising amino acids 2 to 166 of SEQ ID NO: 3.

23. An antibody that specifically binds to a TACI receptor and
inhibits B-cell proliferation and does not inhibit BLyS binding to
25 TACI receptor.

24. The antibody according to claim 1, wherein the antibody is
a monoclonal antibody.

30 25. The antibody according to claim 1, wherein the antibody is
produced by the hybridoma cell line deposited with ATCC as
7B6.15.11 (Accession No. PTA-5000) on February 11, 2003.

35 26. The antibody according to claim 24, wherein the
monoclonal antibody binds to the same epitope as the epitope to
which an antibody that is produced by the hybridoma cell line
deposited with ATCC as 7B6.15.11 (Accession No. PTA-5000) on
February 11, 2003 binds.

27. A monoclonal antibody which binds to the same epitope as the epitope to which the monoclonal antibody produced by the hybridoma cell line deposited with the ATCC as 7B6.15.11 (Accession
5 No. PTA-5000) on February 11, 2003 binds.

28. A hybridoma cell line which produces monoclonal antibody the 7B6 monoclonal antibody and was deposited with ATCC as 7B6.15.11 (Accession No. PTA-5000).
10

29. The monoclonal antibody 7B6 produced by the hybridoma deposited with ATCC as accession number PTA-5000.

30. A monoclonal antibody, comprising an antibody which binds
15 to TACI receptor and competitively inhibits binding of the monoclonal antibody produced by the hybridoma deposited as ATCC PTA-5000 to said TACI receptor.

31. A monoclonal antibody which specifically binds to TACI
20 polypeptide and comprises a sequence derived from the variable domain of an antibody produced by the hybridoma deposited with ATCC as accession number PTA-5000.

32. The antibody of claim 31 which is a chimeric antibody.
25

33. The antibody of claim 31 which is a humanized antibody.

34. The antibody of any one of claims 23, 26, 30 and 31 which
30 is linked to one or more non-proteinaceous polymers selected from the group consisting of polyethylene glycol, polypropylene glycol, and polyoxyalkylene.

35. The antibody of any one of claims 23, 26, 30 and 31 which
35 is linked to a cytotoxic agent or enzyme.

36. The antibody of any one of claims 23, 26, 30 and 31 which is linked to a radioisotope, fluorescent compound or

chemiluminescent compound.

37. The antibody of any one of claims 23, 26, 30 and 31 which is glycosylated.

5

38. The antibody of any one of claims 23, 26, 30 and 31 which is unglycosylated.

39. A method of modulating TACI polypeptide biological activity in mammalian cells, comprising exposing said mammalian cells to the antibody according to any one of claims 23, 26, 30 and 31.

40. A monoclonal antibody which binds to the same epitope as the epitope to which the antibody produced by the 4C7.2.1 hybridoma cell line deposited as ATCC accession number PTA-4999 binds.

41. The 4C7.2.1 hybridoma cell line deposited with ATCC as accession number PTA-4999.

20

42. The monoclonal antibody secreted by the 4C7.2.1 hybridoma deposited with ATCC as accession number PTA-4999.

43. A chimeric anti-TACI antibody which specifically binds to TACI polypeptide and comprises a sequence derived from the variable domain of the antibody secreted by the 4C7.2.1 hybridoma deposited with ATCC as accession number PTA-4999.

44. The anti-TACI antibody of claim 43 which is a humanized antibody.

30

1 AGCATCTCTGA GTRATGAGTG GCCTGGGCGG GAGCAGGGGA GGTGGCCGGA GCGGTGTGGA CCAGGAGGAG CGCTTTCAC AGGGCTGTG GACGGGGTG
 TCTAGGACT CATTACTCAC CGGACCGCGC CTCTCCGCT CCACGGCCT CGGCACACCT GGTCTCTC TCAGAGGTG TCCGGACAC CTCCCCCACC
 1 M S G L G R S R R G G R S R V D Q E E R F P Q G L W T G V
 101 GCTATGAT CTTGCCCGA AGACGATAC TGGATCTC TCTGGGTAC CTGATGTCC TGCARACCA TTTCRACCA TCAGAGCCAG CGCACCTGTG
 CGTACTCTA GACGGGGCT TCTGTCTAG ACCTAGGAG ACACCCCATG GACGTACAGG ACCTTTTGT AAACCTTGT AGCTCTGGT GGTGGACAC
 30 A N R S C P E E Q Y W D P L L G T C M S C K T I C N H Q S Q R T C A
 201 CAGCTTCTG CAGCTACTC AGTTCGCCA AGGACGAGG CAGTTCTAT GACCATCTCC TGAAGGACTG CATCAGCTGT GCTCCCTCT GTGACACAGC
 GTCGAGAC GTCCAGTGAG TCGAGGGCT TCTCGTTCC GTTCAGATA CTGTAGAGG ACTCCCTGAC GTATGACAC CGGAGGTAGA CACTGTCTG
 64 A F C R S L S C R K E Q G K F Y D H L L R D C I S C A S I C G Q H
 301 CCTAAGCAA TGTGCTACT TCTGTAGAA CAGCTCAGG AGCCAGTGA ACCTTCCAC AGCTCAGG AGACAGCGGA GTGAGGAGT TGAACACAT
 GGTTCGTT ACAGTATGA AGACACTCT GTTCAGTCC TGGGTCACT TGAAGGTGG TCTCAGTCC TCTGTGCT CACTCTCTCA ACTTTGTA
 97 P K Q C A Y F C E N K L R S P V N L P P E L R R Q R S G E V E N N
 401 TCAGACACT CGGAGGATA CCAAGATG GAGCAGAGG GCTCAGAGC AGTCCAGCT CTCCGGGGC TGAAGCTGAG TGCAGATCAG GTGGCCCTG
 AGTCTGTGA GCTCTCCAT GGTCTCTAC CTCTGTCTC CGAGTCTTG TTAAGTCTGA GAGGGCCCG ACTTGGACTC AGCTCTAGT CACCGGGAC
 130 S D N S G R Y Q G L E H R G S E A S P A L P G L K L S A D Q V A L V
 501 TCTACAGCAC GCTGGGGCTC TGCCTGTG TGCCTCTCTG CTGCTCTCTG GTGGCGGTG CTTGCTCTCT CAGGAGAGG GGGATCTCT GCTCTGCCA
 AGATCTCTG CGACCCCGAG ACAGACAC CAGCAGAGC GACGAGGAC CACCCACAC V A V A C F L K K R G D P C S C Q
 164 Y S T L G L C L C A V L C C F L V A V A C F L K K R G D P C S C Q
 601 GCGCCCTCA AGGCCCTC AAGTCCGC CAGTCTCC CAGGATCAG CGATGAGG CCGGAGCCT GTGACACAT CCCCCGACC AGTGGAGAC
 CGGGGAGT TCGGGGCG TTTGAGGCG GTTCAGAGG GTCTAGTCC GCTACTCTG GCGTGGGA CACTCTGTA GGGGCTGG TCACTCTG
 197 P R S R P R Q S P A K S S Q D H A M E A G S P V S T S P E P V E T
 701 TGCAGCTCT GCTTCCCTGA GTGAGGGCG CCCACGAGG AGAGCCAGT CAGGCTGG ACCCCGACC CACTTGTG TGAAGGTGG GGGTCCACA
 ACGTCAAGA CGAGGGACT CAGTCCCG CAGTCTCT TCTGGGTCA GTGGGACCC TGGGGCTGG GGTGACACG ACTTCCACC CCGAGGTGT
 230 C S F C F P E C R A P T Q E S A V T P G T P D P T C A G R W G C H T
 801 CAGGACAC AGTCTCTCAG CTTGCCAC ACATCCAGA CAGTGGCTT GCAATGTGT GTGTGCTG CCGAGAGG GGGCAGGT CATATATGG
 GGTCTGTG TCAGGAGTC GAGCGGTG TGTAGGTCT GTACCGAA CCGTACACA CACAGGAG GGTCTCTCC CCGGTCTCAC GTATTTACC
 264 R T T V L Q P C P H I P D S G L G I V C V P A Q E G G P G A O

FIG. 1A

901 GGTGAGGAG GGAAGGAGG AGGAGAGAG ATGAGAGCA GGGAGAGAG AAAGAGAGT GGGAGAGGG GAGAGGATA TGAGGAGA GAGACAGAGG
CCAGTCCCTC CCTTCCCTC TCCCTCTCTC TACCTCTCT TTTCTCTCC CCCCCTCTCC CTCTCTCTAT ACTCTCTCT CTCTGTCTCC
1001 AGGCAGAAAG GGAGAGAAAC AGAGGAGACA GAGAGGAGA GAGAGACAGA GGGAGAGAGA GACAGAGGG AAGAGGGGA GAGAGGAAA GAGGCAGAGA
TCCGCTTTC CCTCTCTTG TCTCCTCTGT CTCTCCCTCT CTCTCTGCT CCGTCTCTCT CTCTCTCCCT TTCTCTCCGT CTCTCCCTTT CTCCGTCTCT
1101 AGGAAAGAGA CAGGCAGAGA AGGAGAGAGG CAGAGAGGGA GAGAGGAGA GAGGAGAGA GGCAGAGAGA CAGAGAGGGA GAGAGGAGA GAGAGAGATA
TCCCTTCTCT GTCCGTCTCT TCCCTCTCTC GTCTCTCCCT CTCTCCGTCT CTCCCTCTCT CCGTCTCTCT GTCTCTCCCT CTCTCCCTGT CTCTCTCTAT
1201 GAGCAGGAGG TCGGGGCACT CTGAGTCCCA GTTCCAGTG CAGCTGTAGG TCGTCATCAC CTAAACACAC GTGCAATAAA GTCCCTCGTC CTGCTGCTCA
CTCGTCTCTC AGCCCGGTGA GACTCAGGGT CAAGGTCAC GTGACATCC AGCAGTAGTG GATTGTGTG CAGTTATTT CAGGAGCAG GACGAGGAGT
1301 CAGCCCCCGA GAGCCCTCC TCCCTGGAGAA TAAACCTTT GCGAGCTGCC CTTCCTCAA AAAAAAAAAA AAAAAA
GTGGGGGCT CTGGGAGG AGGACCTCT ATTTGGAAA CCGTCGACGG GAAGGAGTT TTTTCTTTT TTTTTT

FIG. 1B

HTACI(265) HTACI	10	20	30	40	50
	M S C L G R S R R G G R S R V D Q E E R F P Q G L W T G V A M R S C P E E Q Y W D P L L G T C M S C				
	Y S Q L G R S R R G G R S R V D Q E E R F P Q G L W T G V A M R S C P E E Q Y W D P L L G T C M S C				
HTACI(265) HTACI	60	70	80	90	100
	K T I C N H Q S Q R T C A A F C R S L S C R K E Q G K F Y D H L L R D C I S C A S I C G Q H P K Q C				
	K T I C N H Q S Q R T C A A F C R S L S C R K E Q G K F Y D H L L R D C I S C A S I C G Q H P K Q C				
HTACI(265) HTACI	110	120	130	140	150
	A Y F C E N Y L R S P V N L P P E L R R Q R S G E V E N N S D N S O R Y Q G L E H R O S E A S P A L				
	A Y F C E N K L R S P V N L P P E L R R Q R S G E V E N N S D N S O R Y Q G L E H R O S E A S P A L				
HTACI(265) HTACI	160	170	180	190	200
	P G L K L S A D Q V A L V Y S T L G G L C L C A V L C C F L V A V A C F L K K R O D P C S C Q P R S R				
	P G L K L S A D Q V A L V Y S T L G G L C L C A V L C C F L V A V A C F L K K R O D P C S C Q P R S R				
HTACI(265) HTACI	210	220	230	240	250
	P R Q S P A K S S Q D H A M E A G S P V S T S P E P V E T C S F C F P E C R A P T Q E S A V T P G T				
	P R Q S P A K S S Q D H A M E A G S P V S T S P E P V E T C S F C F P E C R A P T Q E S A V T P G T				
HTACI(265) HTACI	260	270	280	290	300
	P D P T C A G R T T T V L Q P C P H (P D S G L G I V C V P A P R E G)				
	P D P T C A G R T T T V L Q P C P H (P D S G L G I V C V P A Q E G G P C A Z)				

FIG. 1C

1 AAGACTCAA CTTAGAAACT TGAATTAGAT GTGGTATCA AATCCTTAGG TGCGCGGAAG ACACAGACAG CCCCCTTAAG ACCCAGCA GCAGGGGAG
 TTCTGAGTTT GAATCTTTGA ACTTATCTA CACCATRAGT TTAGGARTGC ACGCGGCTTC TGTTCTCTGC GGGGGCATTC TTGGGTGCTT CGTCCGCTTC
 101 TTCATTGTT CCAACATTCT AGCTGCTCTT GCTGCATTG CTCTGGAATT CTGTGAGAGA TATTACTTGT CCTTCCAGGC TGTTCTTTCT GTAGCTCCCT
 AAGTAACAAG AGTTGTAAGA TCGACGAGAA CGACGTAAAC GAGACCTTAA GAGACCTCTCT ATAATGAACA GGRAGGTCCG ACAAGAAAGA CATCGAGGGA
 201 TGTCTTCTT TTGTGATCAT GTTGCAGATG GCTGGGCAGT GCTCCCAAAA TGAATATTTT GACAGTTTGT TGCATGCTTG CATACCTTGT CAACCTTCAT
 ACAAAGAAA ARACTAGTA CAACGCTCTAC CGACCCGTCA CGAGGGTTTT ACTATATAAA CTGTCAACA ACGTACGAAC GTATGGAACA GTTGAAGCTA
 1 Me tLeuGlnMet AlaGlyGlnC ysSerGlnAs nGluTyPhe AspSerLeul euHisAlaCy sileProCys GlnLeuArgC
 301 GTTCTTCTAA TACTCCTCCT CTRACATGTC AGCGTTATTG TAATGCAAGT GTGACCAATT CAGTGRAAGG AACGRAATGC ATTCTCTGGA CCTGTTTGGG
 CRAAGAGATT ATGAGGAGGA GATTGTACAG TCGCRATRAAC ATTACGTTCA CACTGGTTAA GTCACTTTCC TTGCTTACGC TAAGAGACCT GGACAAACCC
 29 ysSerSerAs nPheProPro LeuThrCysG InArgTyCy sAsnAlaSer ValThrAsnS erVallysGl yThrAsnAla IleLeuTript hrCysLeuGl
 401 ACTGAGCTTA ATATTTCTT TGGCAGTTT TGGCTAATG TTTTGTCTAA GGAAGTAAAG CTCTGAAACA TTAAGGAGC AGTTTAAAA CACAGGATCA
 TGACTCGAAT TATTAAAGAA ACCGTCAAAA GCACGATTAC AAAAAGGATT CCTTCTATTG GAGACTTGT ATTTCTCTGC TCRAATTTT GTGTCTTAGT
 62 yLeuSerIeu IleIleSerL euAlaValPh eValLeuMet PheLeuLeuA rGlySileSe rSerGluPro LeuLysAspG luPheLysAs nThrGlySer
 501 GGTCTCTGG GCATGGCTAA CATTGACCTG GAAAGAGCA GGAATGGTGA TGAATATTAT CTTCGGAGAG GCTCGAGTA CACGGTGGAA GAATGCACCT
 CCAGAGGACC CGTACCGATT GTAACCTGAC CTTTCTCTGT CCTGACCACT ACTTAAATAA GAAGGCTCTC CGGAGCTCAT GTGCCACCTT CTTACGTGGA
 95 GlyLeuLeuG lyMetAlaAs nIleAspLeu GluLysSera rgThrGlyAs pGluIleIle LeuProArgG lyLeuGluTy rThrValGlu GluCysThrC
 601 GTGAAGACTG CATCAGAGC AARCCGAAG TCGACTCTGA CCATTGCTTT CCACTCCCAG CTATGGAGA AGGCGCAACC ATTCTTGTCA CCACGAAAC
 CACTTCTGAC GTAGTTCTCG TTTGGCTTCC AGCTGAGACT GGTAACGAAA GGTGAGGTC GATACCTCTT TCCGGTTGG TAAGAACAGT GGTGCTTTG
 129 ysGluAspCy sileLysSer LysProLysV alAspSerAs pHisCysPhe ProLeuProA laMetGluGl uGlyAlaThr IleLeuValt hrThrLysTh
 701 GAATGACTAT TCCAAGAGCC TGCCAGCTGC TTTGAGTGT TTTGAGTGT AGRATCAAT TTCTGCTAGG TAATTAACCA TTTCGACTCG AGCAGTGCCA
 CTTACTGATA ACGTTCTCG ACGGTGACG ARACTCACA TGCCTCTATC TCTTAGTTA AAGACGATCC ATTAATTGGT AAAGCTGAGC TCGTCACGGT
 162 rAsnAspTy rCysLysSerL euProAlaAl aLeuSerAla ThrGluLeG lulySerIl eSerAlaArg OC*
 801 CTTTAAAAAT CTTTGTGTCAG AATAGATGAT GTGTGAGATC TCTTAGGAT GACTGTATTT TTCAGTTGCC GATACAGCTT TTGTCTCTCT AACTGTGGAA
 GAAATTTTAA GAAACAGTC TTATCTACTA CACAGTCTAG AGRATCTTA CTGACATAAA AGTCAACCG CTATGTCGAA AACAGGAGA TTGACACCTT
 901 ACTCTTTATG TTAGATATAT TTCTTAGGT TACTGTTGGG AGCTTAATGG TAGAACTTC CTTGGTTTCA TGATTAAAGT CTTTTTTTT CCTGA
 TGAGGAAATAC AATCTATATA AAGAGATCCA ATGACACCCC TCGAATTACC ATCTTTGAAG GAACCAAAGT ACTAATTTCA GAAAAAATAA GGAAT

FIG. 2

1 ATGGAGAGACT CCACAGAAAG GGGACAGTCA CGCCTTACTT CTTGCCCTTAA GAAAGAGAA GAAATGAAC TGAGGAGTGG TGTTCCTATC CTCACAGGGA
 TACTACTGA GGTGTCTTTC CCTGCTCACT GCGGAATGA GAACGGAAT CTTTCTCTT CTTACTTTG ACTTCCTCAG ACAAGGTAG GAGGTGCTCT
 1 M D D S T E R E Q S R L T S C L K K R E E M K L K E C V S I L P R K
 101 AGGAAGGCC CTCTGTCCGA TCCTCCAAAG ACGGAAGCT GCTGGCTGCA ACCTTGCTGC TGSCACTGCT GTCTTGCTGC CTCACGGTGG TGTCTTTCTA
 TCCTTTCCGG GAGACAGGCT AGGAGGTTTC TGCTTTTGA CGACCGACCT TGACAGCAG AGCTGACCA CAGACGACGG GAGTGCACCC ACAGAAGAT
 35 E S P S V R S S K D G K L L A A T L L L A L L S C C L T V V S F Y
 201 CCAGGTGGCC GCCTGCGAG GGGACCTGCC CAGCCTCCGG GCHAGCTGCG AGGCCACCA CCGGAGAGG CTGCCAGCAG GACGAGGAG CCCGAGGCC
 GGTCCACGG CGGACGTTTC CCTGAGCGG GTCCGAGCG CGTCTGAGG TCOCGGTGGT GCGCTCTTC GAGGTCTGC CTGCTCCTCG GGGGTCCGG
 68 Q V A A L Q G D L A S L R A E L Q G H A E K L P A G A G A P K A
 301 GGCTTGGAG AGCTCCAGC TGTACCGCG GCACTGAAA TCCTTGNACC ACCAGCTCCA GAGAGAGGCA ACTCCAGTCA GACACGAGA AATAGCGGTG
 CCGACCTCC TTGAGGTGCG ACAGTGGCG CCTGACTTTT AGAAGCTTGG TGGTCAGGT CCTCTTCCGT TGAGGTCACT CTTGTGCTCT TTATTGCTC
 101 G L E E A P A V T A G L K I F E P P A P G E G N S S Q N S R N K R A
 401 CCGTTCRGG TCCAGAGAA ACAGTCATC ARGCTGCTT GCACTGATT GCAGACAGTG AACACCCAAC TATACAAAAG GATCTTACA CATTTGTTCC
 GCGAGTCCC AGGTCTTCTT TGTCACTGAG TTCTGACGAA CGTTGACTAA CGTCTGTCAC TTGTGGTGG ATATGTTTTT CCTAGATGT GTAACAGAGG
 135 V Q G P E E T V T Q D C L Q L I A D S E T P T I Q K G S Y T F V P
 501 ATGGCTTCTC AGCTTTAAA GGGGAGTGC CCTAGAGAA AAGAGATA ATATATTGCT CAAGAACT GGTACTTTT TTATATATGG TCAGGTTTTA
 TACCGAGAG TCGAATTTT CCCCTCACG GATCTTCTT TTCTCTTAT TTATATACCA GTTCTTTTGA CCATGAAA AAATATATACC AGTCCAAAT
 168 W L L S F K R G S A L E E K E N K I L V K E T G Y F I Y G Q V L
 601 TATCTGATA AGACCTACG CATGGACAT CTAAATCAGA GAGAGAGGT CCATGCTTT CCATGCTGAT TGACTCTGCT GACTTTGTTT CGATGATTC
 ATATGACTAT TCTGATGCG GTACCTGTA GATTAAGTCT CCTTCTTCCA GTACAGAA COCCTACTTA ACTGAGCA CTGAAACAAA GCTACATAG
 201 Y T D K T Y A M G H L I Q R K K V H V F G D E L S L V T L F R C I Q
 701 AATATAGCC TGAACACTA CCCAATATT CTGCTATT CAGTGGCATT GCAAACTGG AAGAGAGGA TGAATCCAA CTGCAATAC CAAGAGAAA
 TTTATACGG ACTTTGTGAT GGGTATATA GAGCATAAG TCGACCGTAA CGTTTGACC TTCTCTCTCT ACTTGAGGT GAACGTATG GTTCTCTTT
 235 N M P E T L P N N S C Y S A G I A K L E E G D E L Q L A I P R E N
 801 TGCACAAATA TCACCTGGATG GAGATGTCAC ATTTTGTGCT GCATGAAC TCGTGGA
 ACGTGTATT AGTGACCTAC CTCTACAGTG TAABAAACCA CGTAAGTTTG ACGACACT
 268 A Q I S L D G D V T F F G A L K L L O

FIG. 3

1 GGTACGAGGC TTCTAGAGG GACTGGACC TAACTCTCTT GAGCTGAGG GAGGGTGGAG GGTTCTCAGG CAAAGCTGGC CCAAGCAGGG AGTGCCAGGA
 CCAAGCTCG AGAGTCTCC CTGACTTCG ATTAAGAGGA CTCGACTCC CTCACACTC CAGAGTTCC GTTGGACCG GGTTGCTGCG TCACGGTCTT
 101 GCACTAACAG TACCTTAGC TTGCTTTCTT CTTCTCTCTT AAGTTCCTT TTAATTTCTT TTGGTAACA ACCTTCTCC CTCTCTGACC
 CTTGATCTC ATGGGAATCG AACGAAAGGA GGAGGAGGA AATAAAGG TTCAAGGAA AATAAAGAGG AACGATTTGT TGGAAAGAGG GAGACGCGG
 201 ACTGCCCGTA CCTTACCG CCGGCCAC CCTTCTTAC TCTTCTTAC CCACTCTTG AACACACAG TGTGGCAGG GTCCACAGCT CATGCCAGCC TCATCTCTCT
 TACCGGGCAT GGGATGGGC GGGCGGCTGG AGGACGATG GGTGAGATC TTGCTGTCG ACHACCGTCC CAGGGGTGCG GTACGCTCGG ACTAGAGGAA
 M P A S S P F
 301 TCTTGTCTAGC CCCCAGAGG CTTCCAGGA ACATGGGGG CCAATCAGA GAGCGGCGAC TCTCAGTTCC CTTCTGTTG AGTTGGGGG CAGCTCTGGG
 AGRACGATCG GGGGTTTCC GGAGTCTCGT TGTACCCCGC GGTGAGTCTT TGTACGCTG AGAGTCAAG GAGACCAAC TCACCCCGC GTCCGAGAGCC
 8 L L A P K G P P G N M G G P V R E P A L S V A L W L S W G A A L G
 401 GGGCTGGCT TGTGCAATG CTCTGCTGAC CCAACAAACA GAGCTGCAGA GCCTCAGGAG AGAGGTGAGC GGGCTGCRGG GAGACGAGG CCGCTCCAGG
 CCGGACCGA ACACGATAC GAGAGGACTG GGTGCTTTGT CTCGAGCTCT CCGAGTCTC TCTCCACTCG GCGACGCTCC CTTCTCTCTC GGGAGGGTC
 41 A V A C A M A L L T Q Q T E L Q S L R R E V S R L Q G T G G P S Q
 501 AATGGGAG GGTATCCCTG GCAGATCTC CCGAGCAGA GTTCCGATC CTTGAGCC CTTGAGATG GGGAGAGATC CCGAAGAGG AGAGCAGTGC
 TTACCCCTTC CCAAGGGAC CGTCTCAGG GGCCTGCTCT CAGGCTAGG GAGCTTCCG ACCCTCTTAC CCTCTCTAG GGCCTTTTCC TCTCGTCTAG
 74 N G E G Y P W Q S L P E Q S S D A L E A W E N G E R S R K R R A V L
 601 TCACCCAAA ACAGAGAG CAGCACTCTG TCCTGCACTT GGTCCACTT AACGCCACT CCAAGGATGA CTCGATGTG ACRAGGTGA TGTGGCAGCC
 AGTGGGTTT TGTCTCTTC GTCGTGAGC AGGAGGTGA CCAAGGGTAA TTGGGTGGA GGTCTCTACT GAGGTATAC TGTCTCTACT ACACGTTGG
 108 T Q K Q K K Q H S V L H L V P I N A T S K D D S D V T E V M W Q P
 701 AGCTCTTAGG CGTGGGAGG GCCTACAGG CCAAGCATAT GGTTCCGAA TCCAGGATG TGGAGTTTAT CTGCTGTATA GCGAGTCTT GTTCAAGAC
 TCGAATCC GCACCTCTC CCGATGTCG GGTCTCTATA CCAAGGCTT AGGTCTTACG ACCTCAATA GAGACATAT CCGTCCAGG CAAAGTTCTG
 141 A L R R G R G L Q A Q G Y G V R I Q D A G V Y L L Y S Q V L F Q D
 801 GTGACTTTCA CCAAGGTC GGTGTGTCT CAGAGGCG CAGAGGCA GAGACTCTA TTCGATGTA TAAAGATAT GCGCTCCAC CCGGACCGGG
 CACTGAAGT GTTACCGAGT CCAACACAGA GCTCTCCGG TTCTTCCGT CTTCTGAT AGGCTACAT ATTCTCATA CCGGAGGTG GCGCTGGGCC
 174 V T F T M G Q V V S R E G Q G R Q E T L F R C I R S M P S H P D R A
 901 CCTACACAG CTGCTATAGC GCAGGTGCT TCAATTACA CCAAGGGAT ATTCTGATG TCATAATTC CCGGCAAGG GCGAATCTA ACCTCTCTCC
 GAGTGTGTC GAGGATATCG CGTCCACAGA AGGTAAATGT GGTTCCTTA TAAAGTCAAG AGTATTAAG GCGCGTCTC CGCTTGAAT TGGAGAGAGG
 208 Y N S C Y S A G V F H L H Q G D I L S V I I P R A R A K L N L S P

FIG. 4A

1001 ACATGGAACC TTCCTGGGGT TTGTGAACCT GTGATTGTGT TATAAAGT GGCTCCAGC TTGGAAGCC AGGTGGGTA CATACTGGAG ACGCCAGA
TGTAACCTGG AAGGCCCA AACACTTGA CACTAACACA ATATTTTCA CGAGGGTCG AACCTTCTGG TCCACCCAT GTATGACCTC TGTGGTCT
241 H G T F L G F V K L O
1101 GCTGCTATA TAAGGAGAG GGAATGTGCA GGAACAGAGG CATCTCCTG GGTTCGCTC CCGTTCCTC ACTTTCCTT TTTTCATCCC ACCCCCTAGA
CGACTCATAT ATTTCTCTC CCTTACAGT CCTTGTCTCC GTAGAGGAC CCAACCGAG GGGCHAGGAG TGAAGGGA AAGTAAGGG TGGGGGATCT
1201 CTTTGATTT ACGGATATCT TGCTTCTGTT CCCCATGGAG CTCGGAATTC TTGCTGTGT GTAGATGAGG GGGGGGGAC GGGCCCCAGG CATTTGTTGAG
GAACTAATA TGCTATAGA ACGAGACAA GGGGTACCTC GAGGCTTARG AACGCACACA CATCTACTCC CCGCCCCCTG CCGGGGTCC GTACCAAGTC
1301 ACCTGGTCGG GGGCCACTGG AAGCTCCAG AACAGCACCA CCATCTTA
TGAACAGGCC CCGGGTGACC TTCGTAGGTC TTGTCGTGGT GGTAAGAT

FIG. 4B

TACIs

agcatcctgagtaATGAGTGGCCTGGGCCGGAGCAGGCCGAGGTGGCCGGAGCCGTGTGGACCAGG
AGGAGCGCtGGTCACTCAGCTGCCGCAAGGAGCAAGGCAAGTTCTATGACCATCTCCTGAGGGAC
TGCATCAGCTGTGCCTCCATCTGTGGACAGCACCCCTAAGCAATGTGCATACTTCTGTGAGAACAA
GCTCAGGAGCCCAGTGAACCTTCCACCAGAGCTCAGGAGACAGCGGAGTGGAGAAGTTGAAAACA
ATTGAGACAACTCGGGAAGGTACCAAGGATTGGAGCACAGAGGCTCAGAAGCAAGTCCAGCTCTC
CCGGGGCTGAAGCTGAGTGCAGATCAGGTGGCCCTGGTCTACAGCACGCTGGGGCTCTGCCTGTG
TGCCGTCTCTGCTGCTTCTGGTGGCGGTGGCCTGCTTCCTCAAGAAGAGGGGGGATCCCTGCT
CCTGCCAGCCCCGCTCAAGGCCCGTCAAAGTCCGGCCAAGTCTTCCAGGATCACGCGATGGAA
GCCGGCAGCCCTGTGAGCACATCCCCGAGCCAGTGGAGACCTGCAGCTTCTGCTTCCCTGAGTG
CAGGGCGCCACGCAGGAGAGCGCAGTCACGCCCTGGGACCCCCGACCCCACTTGTGCTGGAAGGT
GGGGGTGCCACACCAGGACCACAGTCTTGCAGCCTTGCCCACACATECCAGACAGTGGCCTTGGC
ATTGTGTGTGTGCTGCCAGGAGGGGGGCCAGGTGCATAAatgggggtcagggagggaaagga
ggagggagagagatggagaggaggggagagagaaagagaggtggggagaggggagagagatatga
ggagagagagacagaggaggcagaaaggagagaaaacagaggagacagagaggggagagagagaca
gagggagagagagacagaggggaagagaggcagagagggaaagaggcagagaaggaaagagacag
gcagagaaggagagagggcagagagggagagaggcagagaggggagagaggcagagagacagagagg
gagagaggggacagagagagatagagcaggaggtcggggcactctgagtcaccagtcccagtgag
ctgtaggtcgatcacctaaccacacgtgcaataaagtcctcgtgcctgctgctcacagcccc
gagagccccctcctcctggagaataaaaccttggcagctgcccttcctcaaaaaaaaaaaaaaaaa
aaaa

Fig. 5A

TACIs :

MSG LGRSRRGGRSRVDQEERWSLSRKEQGKFYDHLLRDCISCASICGQHHPKQCAYFCENKLRSF
VNLPPPELRRQRSGEVENNSDNGRYQGLEHRGSEASPALFGLKLSAQVALVYSTLGLCLCAVLC
CFLVAVACFLKKRGDPCSCQPRSRPRQSPAKSSQDHAMEAGSPVSTSPPEVETCSFCFPECRAPT
QESAVTPGTPDFTCAGRWGCHTRTTVLQFCPHIPDSGLGIVCVPAQEGGPG

Fig. 5B

human BR3:

cgtcggcaccATGAGGCGAGGGCCCCGGAGCCTGCGGGGCAGGGACGCGCCAGCCCCACGCCCT
GCGTCCCGGCCGAGTGCTTCGACCTGCTGGTCCGCCACTGCGTGGCCTGCGGGCTCCTGCGCACG
CCGCGGCCGAAACCGGCCGGGGCCAGCAGCCCTGCGCCCAGGACGGCGCTGCAGCCGCAGGAGTC
GGTGGGCGCGGGGGCCGGCGAGGCGGCGCTGCCCTGCCGGGCTGCTCTTTGGCGCCCCGCGC
TGCTGGGCCTGGCACTGGTCCTGGCGCTGGTCCTGGTGGGTCTGGTGAGCTGGAGGCGGCGACAG
CGGCGGCTTCGCGGCGCGTCCTCCGCAGAGGCCCCGACGGAGACAAGGACGCCCCAGAGCCCT
GGACAAGGTCATCATTCTGTCTCCGGGAATCTCTGATGCCACAGCTCCTGCCTGGCCTCCTCCTG
GGGAAGACCCAGGAACCACCCACCTGGCCACAGTGTCCCTGTGCCAGCCACAGAGCTGGGCTCC
ACTGAACCTGGTGACCACCAAGACGGCCGGCCCTGAGCAACAATAGcagggagccggcaggaggtg
gccccctgccc

Fig. 6A

BR3:

MRRGPRSLRGRDAFAPTPCVPAECFDLLVRHCVACGLLRTPRPKPAGASSPAPRTALQPQESVGA
GAGEAALPLPGLLFGAPALLGLALVLALVLVLVGLVSWRRRQRRRLRGASSAEAPDGDKDAPEPLDKV
IILSPGISDATAPAWPPPGEDPGTTPFGHSVPVPATELGSTELVTTKTAGEQQ

Fig. 6B

PRO	XXXXXXXXXXXXXXXXXX	(Length = 15 amino acids)
Comparison Protein	XXXXXXYYYYYYY	(Length = 12 amino acids)

% amino acid sequence identity =

(the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide) =

5 divided by 15 = 33.3%

FIG. 7A

PRO	XXXXXXXXXXXX	(Length = 10 amino acids)
Comparison Protein	XXXXXXYYYYYYZZYZ	(Length = 15 amino acids)

% amino acid sequence identity =

(the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide) =

5 divided by 10 = 50%

FIG. 7B

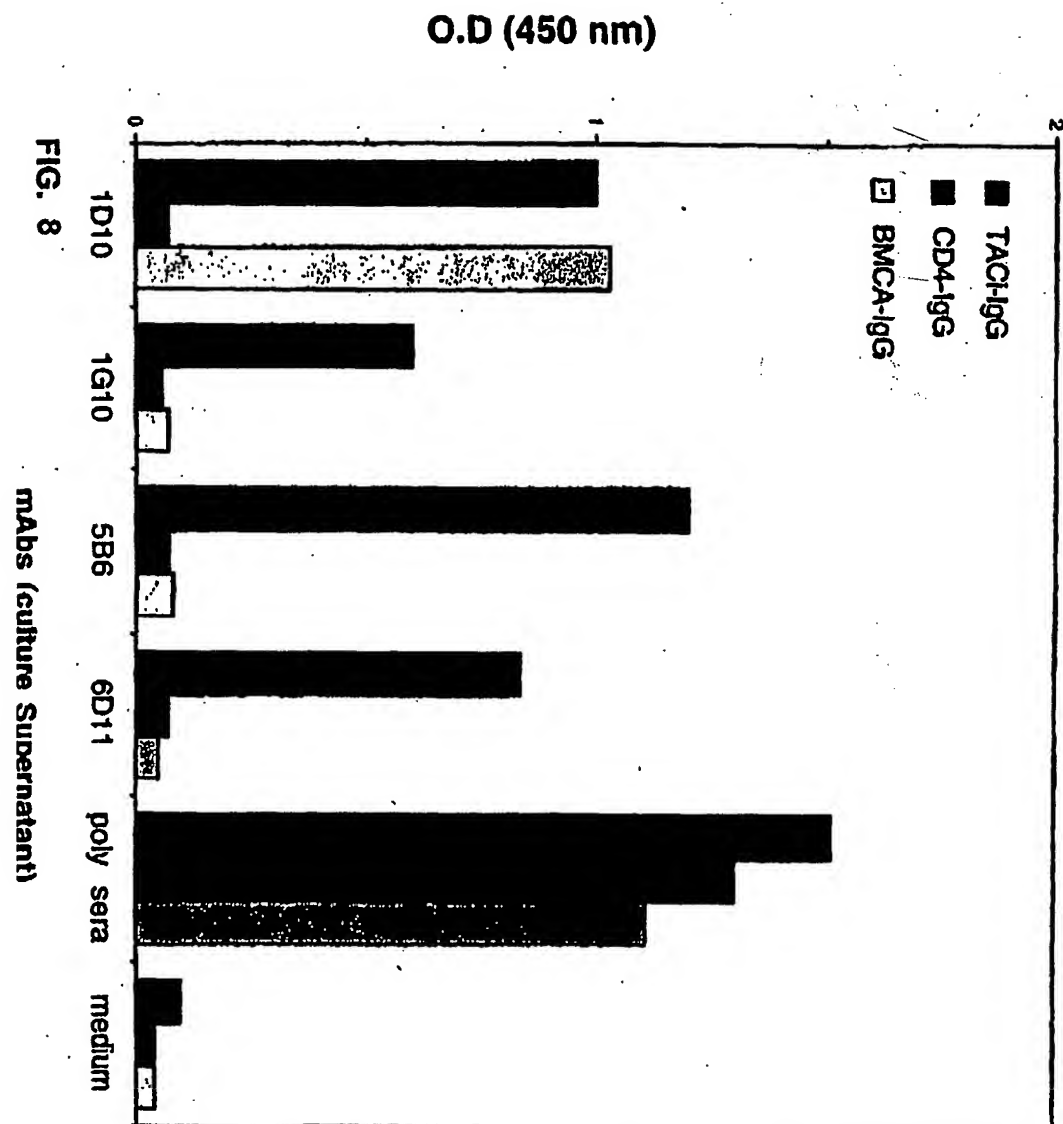


FIG. 8

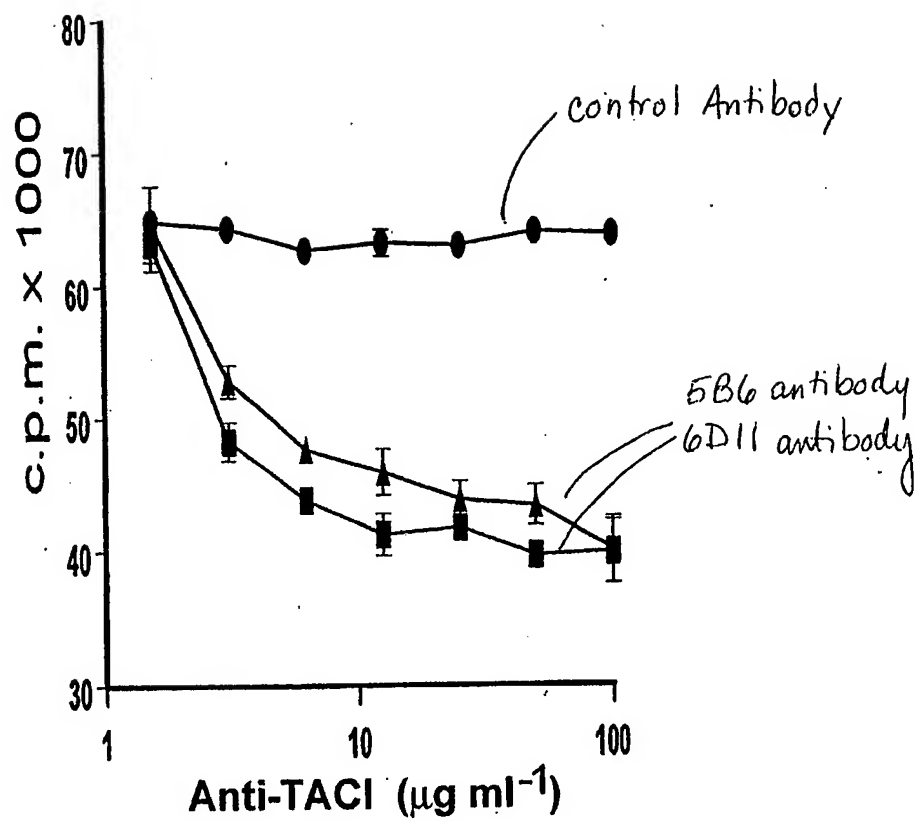


Figure 9

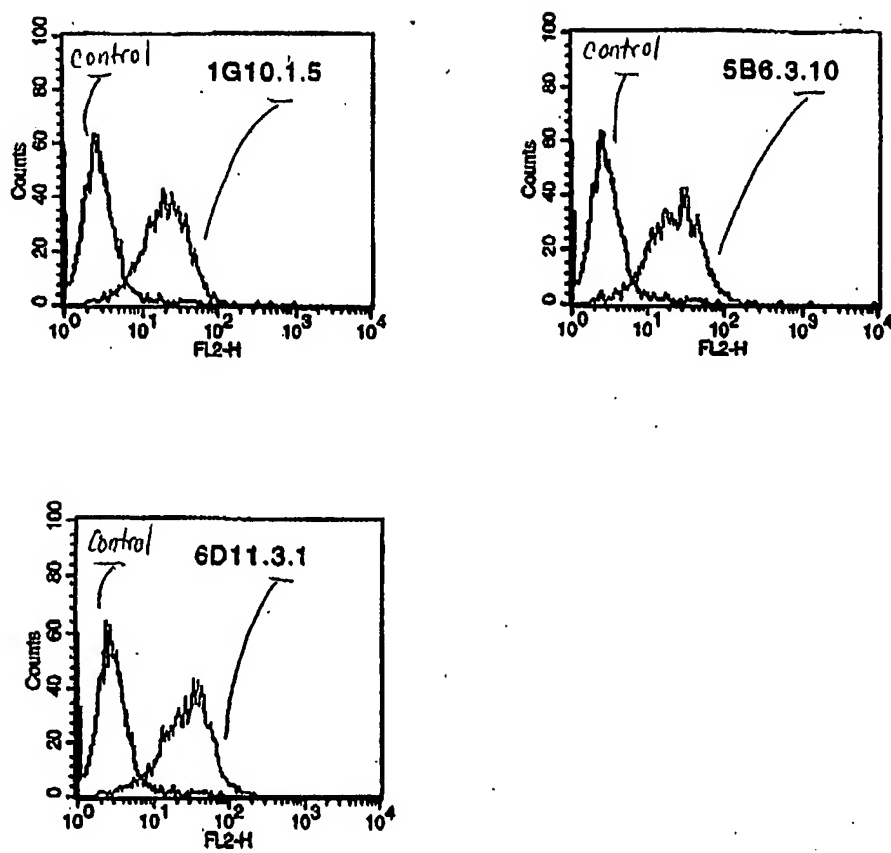


FIG. 10

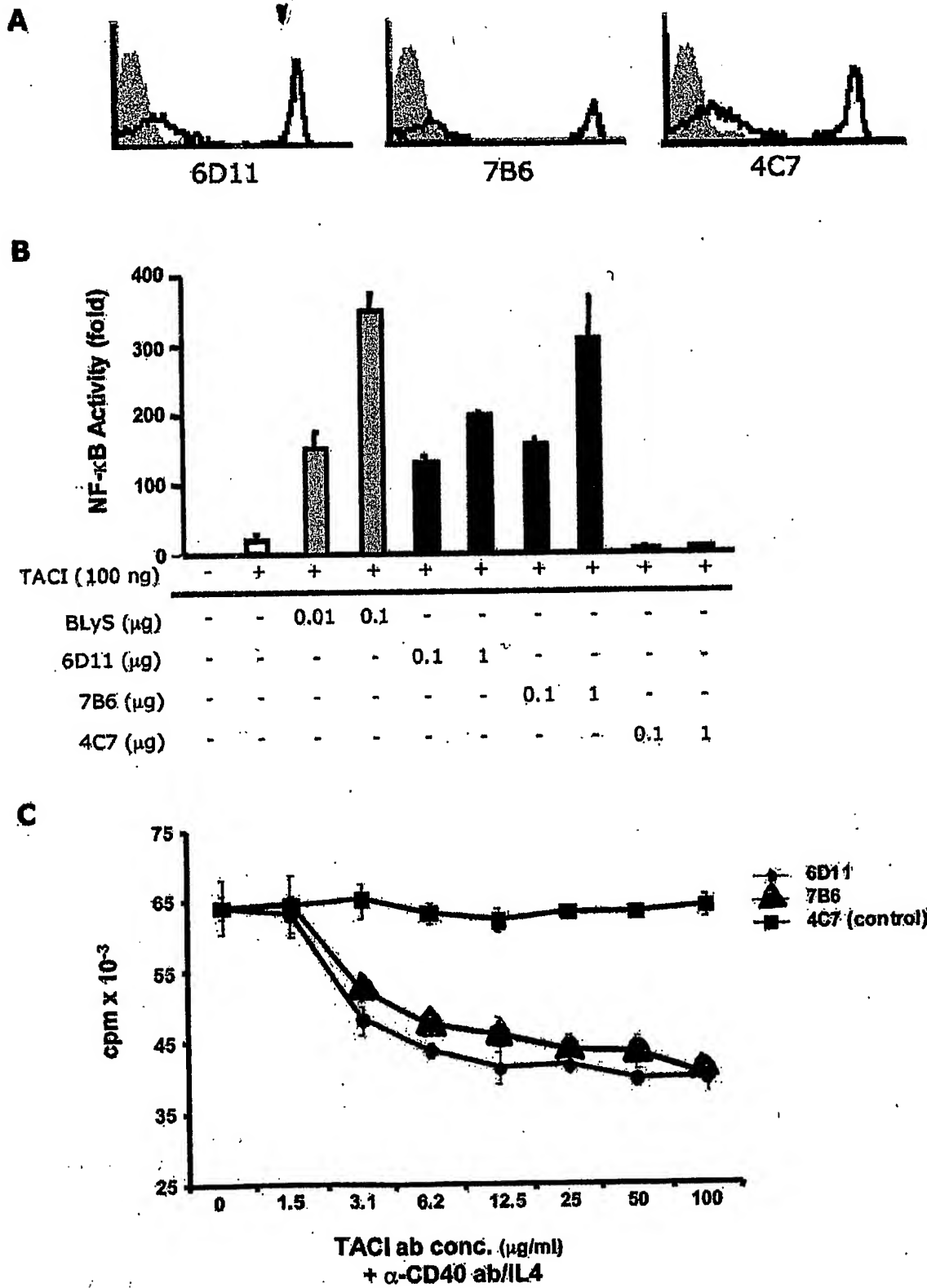


FIG. 11